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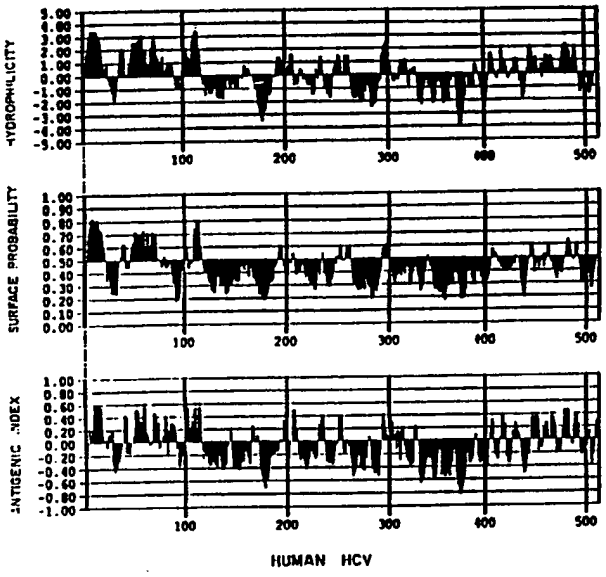
(54) Title: BASIC STRUCTURAL IMMUNOGENIC POLYPEPTIDES HAVING EPITOPES FOR HCV, ANTIBODIES, POLYNUCLEOTIDE SEQUENCES, VACCINES AND METHODS

(57) Abstract

Novel basic immunogenic polypeptides having epitopes for HCV are disclosed. The novel basic immunogenic polypeptides are truncated polypeptides derived from the structural region of a human HCV isolate. Two preferred novel basic immunogenic polypeptides are designated as FGB1 and FGB2. The FGB1 and FGB2 polypeptides are believed to be derived from near the N- and C-terminals, respectively, of a putative (C) protein of HCV. In an ELISA, eight serum samples reactive for anti-C100 HCV antibody (EIA, Ortho/Chiron), recombinant immunoblot assay I (RIBA I, Ortho/Chiron) and neutralization (Neut., Abbot Labs.) contained antibodies to the FGB1 and FGB2 polypeptides. In addition, the FGB1 and FGB2 polypeptides and RIBA I were non-reactive with twelve serum samples which were reactive with EIA. Still further, two serum samples from a patient clinically diagnosed as NANBH, which were non-reactive with EIA and RIBA I, were reactive with FGB1 and/or FGB2. Novel polynucleotide sequences encoding the basic immunogenic polypeptides are also disclosed. The present invention further includes the application of these new sequences, polypeptides, and antibodies raised against the novel polypeptides in HCV detection, such as immunoassays, diagnostics, PCR technology and gene therapy. Included within the invention also are novel immunogenic HCV polypeptides encoded within recombinant expression systems and clones, novel transformants, novel probes, novel methods for producing the immunogenic HCV polypeptides, novel anti-idiotype antibodies, novel antisense polynucleotides, and novel kits.



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BASIC STRUCTURAL IMMUNOGENIC POLYPEPTIDES HAVING EPITOPES FOR HCV, ANTIBODIES, POLYNUCLEOTIDE SEQUENCES, VACCINES AND METHODS

Related Applications

This is a continuation-in-part application of U.S. patent application, Serial No. 07,369,809, filed on January 14, 1991.

Field of the Invention

The present invention relates to biological materials for detecting hepatitis C virus (HCV) and preventing and/or treating HCV disease. More particularly, the instant invention relates to truncated, basic structural immunogenic polypeptides having epitopes for HCV, to polynucleotide sequences encoding the HCV polypeptides and antisense polynucleotide sequences derived therefrom, to antibodies raised against the HCV polypeptides, to anti-idiotype antibodies raised against antibodies to the HCV polypeptides, and HCV vaccines. These biological materials are believed to be effective as screening agents for HCV as well as for acute and chronic HCV infection, and as protective agents against HCV disease.

Background

Hepatitis is the medical term for inflammation or disease of the liver. Viral hepatitis signifies liver inflammation or disease induced by an infecting hepatotropic virus, and is a major worldwide public health problem. At present, five specific hepatotropic viruses involved in this disease have been isolated and characterized. They are identified as hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), hepatitis D virus (HDV) and hepatitis E virus (HEV). These hepatotropic viruses are believed to cause hepatitis, resulting in illnesses characterized by fever, nausea, vomiting, anorexia (loss of appetite) and, in some instances, jaundice and even death.

In the less severe and more ordinary cases, patients infected with a hepatitis virus normally experience nausea, vomiting, loss of appetite and fever. Jaundice with the appearance of bilirubin in the urine and/or death are uncommon. The symptoms associated with the mild form of hepatitis typically continue for approximately 2-6 weeks and the damage to the liver is normally restored within 8-12 weeks following the prodromal period. While complete recovery occurs in most cases, weariness and fatigue may continue for several months even after the symptoms have disappeared. In the most severe cases of

viral hepatitis, the prognosis is much more serious. Generally, the attacks are sudden and the longevity of the illnesses is very acute. The patients infected with this form of the disease usually experience vomiting, severe jaundice, bilirubin in the urine, delirium, coma and death.

Since at least as early as 1973, HAV and HBV have been detected and partially characterized, and reliable diagnostic assays for their detection and diagnosis have been available. Notwithstanding this advancement in the area of viral hepatitis, the continual appearance of hepatitis in patients negative for HAV and HBV led researchers and the medical community to believe that other hepatotropic agents existed. It was learned through research dating back to as early as about 1974 that these unknown hepatotropic agents principally spread through either fecal/oral routes or blood, and they came to be known as non-A, non-B hepatitis (NANBH) agents.

Over the past two years, intense research relative to the NANBH agents has lead to the isolation and identification of the fourth and fifth hepatotropic viruses, namely, HCV and HEV, respectively. See Choo, Q.-L. et al.: Science, 244:359-362 (1989) and Reyes, G.R. et al.: Science, 247:1335-1339 (1990). HCV is believed to cause the majority (at least about 90%) of the parenterally or blood

transmitted NANBH whereas HEV is believed to be responsible for the transmission of enteric (fecal/oral) NANBH. Like HEV, HAV is principally spread by the fecal/oral routes, whereas HBV, HCV and HDV are transmitted principally by the parenteral routes and blood or blood derived products. Even though all five of these hepatotropic viruses apparently are somewhat related, only HDV is unable to grow and replicate on its own. HDV requires the co-presence of HBV for its replication in humans and therefore is found only in patients infected with HBV. Moreover, HDV infection in the presence of HBV (co-infection or super-infection) is believed to cause the most severe form of viral hepatitis. A high percentage of super-infection cases end in death within a few months from the onset of the disease.

While complete recovery normally occurs in HAV and HEV cases and in over 85% of HBV cases, progression to chronic hepatitis from acute HCV is observed in about 25-55% of HCV cases. Moreover, HCV viruses are known to cause serious liver disease such as cirrhosis of the liver, non-established malignant hepatoma (liver cancer) and the like.

Clinical diagnosis of HCV (parenteral NANBH) has been accomplished heretofore primarily by the exclusion of other hepatitis human viruses such as HAV and HBV. Among the methods used hitherto to detect

putative HCV antigens and antibodies are agar-gel diffusion, counter immunoelectrophoresis, immunofluorescence microscopy, immune electron microscopy, radioimmuno assay, and enzyme-linked immunoabsorbent assay (ELISA). However, none of these assays heretofore available has proved to be sufficiently sensitive, specific, and reproducible to be used as reliable diagnostic tests for HCV, especially acute HCV infections, or for screening blood and blood derived products for HCV contamination.

For example, one present method utilized to detect HCV involves the detection of anti-C100 antibody to the non-structural region of HCV, i.e., C100 protein (antigen). This is accomplished by a commercially available kit distributed by Ortho Diagnostics under the brand name Ortho HCV Ab ELISA test. Unfortunately, it has been found that this assay for detecting HCV cannot identify all blood samples containing HCV, especially those contaminated with acute HCV infections. For instance, it has been reported that this assay detects false positives approximately 40-60% of the time. See Mimms, L. et al.: The Lancet, (336):1590-1591 (Dec. 22/29, 1990); Zuck, T.F. et al.: Transfusion, 30(8):759-761 (1990); Getzug, T. et al.: AASLD Abstracts of Papers. Hepatology, 12(4) Pt.2, Abstract No. 32, p. 845 (Oct., 1990); and Attard, L. et al.: JOHEEC, (11) Supplement

2, Abstract No. 10, S3 (1990). But see Baldi, M., et al.: JOHEEC, (11) Supplement 2, Abstract No. 16, S4 (1990). It also has been reported that the Ortho HCV Ab ELISA assay fails to detect HCV infection approximately 10% of the time. See Getzug, T. et al.: AASLD Abstracts of Papers. Hepatology, 12(4) Pt.2, Abstract No. 32, p. 845 (Oct., 1990).

In another present method utilized to detect HCV, it involves the detection of anti-C100 antibody to the non-structural HCV C100 protein (antigen). This is accomplished by a methodology developed by Ortho/Chiron and is referred to as recombinant immunoblot assay I (RIBA I). While the RIBA I assay for detecting HCV has been found to be more reliable than the Ortho HCV Ab ELISA test, it nevertheless is a qualitative test which is believed to be indeterminant for a significant number of HCV antibody containing samples. See, for example, Alberti, A. et al.: JOHEEC, (11) Supplement 2, Abstract No. 3, S1 (1990).

In yet another present method utilized to detect HCV, it involves the indirect detection of anti-HCV antibody. This is accomplished by a methodology developed by Abbott and is referred to as a neutralization assay. Unfortunately, the neutralization assay for detecting HCV is believed to be unreliable because the test utilizes only a portion of HCV, i.e., the non-structural C100 protein (antigen), while

a true HCV neutralization assay requires the utilization of the infectious particle, i.e., the virion, of HCV.

In still another present method utilized to detect HCV, it involves the detection of HCV-RNA in sera in chronic NANBH by the polymerase chain reaction (PCR) using specific HCV synthetic oligonucleotides. Bartolome, J. et al.: JOHEEC, (11) Supplement 2, Abstract No. 18, S5 (1990). Unfortunately, Bartolome, J. et al. report therein that this assay detects HCV RNA in only 44% of the anti-HCV chronic hepatitis sera, and that there remains the need for a more sensitive test to detect HCV infection.

Presently, the more reliable means to diagnose HCV (parenteral NANBH) infection is by biopsy and differential diagnosis. Whether this form of NANBH (HCV) is acute or chronic may be determined by examining the liver tissue by biopsy according to the criterion described in Sheila Sherlock, Diseases of the Liver and Biliary System, eighth edition, published by Blackwell Scientific Publications, London, pp. 326-333 (1989). Unfortunately, liver biopsies are expensive, painful and may lead to patient complications. Moreover, this technique for diagnosing HCV obviously is inapplicable for screening blood and blood derived products.

Because present tests for the detection of HCV (parenteral NANBH) are ineffective, time consuming, expensive and/or complex, blood and blood products remain potential sources for transmission of HCV (parenteral NANBH) to recipients. In the 1970s, the frequency of post-transfusion NANBH ranged from approximately 7% to 12%. However, in the 1980s, improvements in transfusion medicine, due to the necessity to screen blood supplies for antibody to human immunodeficiency virus (HIV), has significantly reduced the frequency of post-transfusion hepatitis to approximately 5%. See Dienstag, J.L.: Gastroenterology, 99:1177-1180 (1990). Notwithstanding this reduction in the frequency of post-transfusion hepatitis, it is presently believed that HCV (parenteral NANBH) accounts for up to 90% of these cases, as indicated hereinbefore. It is estimated that in the USA alone, HCV (parenteral NANBH) affects about 200,000-300,000 people annually.

For these reasons, there is a significant demand for sensitive, specific methods for inexpensively and effectively screening and identifying carriers of both acute and chronic HCV infections and HCV contaminated blood or blood derived products. Patient care as well as the prevention of transmission of HCV by blood and blood products or by close personal contact require reliable screening, diagnostic

and prognostic tools to reliably detect nucleic acids, antigens and antibodies related to HCV. Moreover, there is an urgent need for effective vaccines and immunotherapeutic agents for the prevention and/or treatment of HCV disease.

Summary of the Invention

In brief, the present invention alleviates and overcomes certain of the above-referenced problems and shortcomings of the present state of the HCV art through the discovery of novel immunogenic HCV polypeptides, each having epitopes unique to HCV. The uniqueness of the epitopes have been determined by their immunological reactivity with anti-HCV antibodies and lack of immunological reactivity with antibodies to HBV.

The novel immunogenic HCV polypeptides of the present invention are truncated structural polypeptides which have been discovered from the structural region of a human HCV genome upon analysis of a putative amino acid sequence translated therefrom. The amino acid sequences of the novel immunogenic HCV polypeptides are believed to contain at least about 15 amino acid residues and more preferably at least about 17 amino acid residues, and lie within the first four percent (4%) of the putative amino acid sequence for the human HCV polyprotein. The novel immunogenic HCV

polypeptides of the instant invention are basic polypeptides naving an estimated pI on the order of about 12, and are believed to include at least about 7 amino acid residues (45%) in their Chou-Fasman (CF) turns, and more particularly between about 7 and 17 amino acid residues (45-100%) in their CF turns. The calculated molecular weights of the novel immunogenic HCV polypeptides of the present invention are at least about 1500 and more particularly in the range of between about 1500 and about 2500. The novel immunogenic HCV polypeptides of the instant invention are further characterized as having in their amino acid sequences at least about 4 arginine amino acid residues (25%) and more particularly between about 4 and 7 (25-47%) arginine amino acid residues, at least about 11 hydrophillic amino acid residues (70%) and more particularly between about 11 and 14 hydrophillic amino acid residues (70-87%), and at least about 5 polar amino acid residues (30%) and more particularly between about 5 and 8 polar amino acid residues (30-53%). Consistent with the estimated pI, the novel immunogenic HCV polypeptides of the instant invention include within their amino acid sequences at least about 5 basic amino acid residues (30%) and more preferably between about 5 and 7 basic amino acid residues (30-47%).

Two novel preferred immunogenic HCV polypeptides of the present invention are designated as FGB1 and FGB2. The FGB1 polypeptide has a calculated molecular weight of about 1908, includes 15 amino acids and has the following sequence:

Lys - Pro - Gln - Arg - Lys - Thr - Lys - Arg - Asn - Thr - Asn - Arg - Arg - Pro - Gln.

The FGB2 polypeptide has a calculated molecular weight of about 1811, also includes 15 amino acids and has the following sequence:

Ser - Arg - Pro - Ser - Trp - Gly - Pro - Thr - Asp - Pro - Arg - Arg - Arg - Ser - Arg.

The above-recited amino acid sequences for the novel FGB1 and FGB2 polypeptides are believed to be truncated structural segments derived from a putative amino acid sequence of a human HCV capsid protein which is believed to be encoded within the first 4% of codons following the predicted ATG initiation codon of a human HCV polyprotein. It is further believed that the amino acid sequence for the novel FGB1 polypeptide lies within the putative amino acid sequence for the human HCV capsid protein near its $-NH_2$ terminal end whereas the amino acid sequence for the novel FGB2 polypeptide lies within the putative amino acid sequence for the human HCV capsid protein near its -COOH terminal end.

The FGB1 and FGB2 polypeptides may also include, for example, an additional amino acid residue, such as cysteine, or a cystine moiety at the $-NH_2$ terminal end in order to facilitate for instance the conjugation of carrier polypeptides or molecules, such as keyhole limpet hemocyanin (KLH) or ovalbumin, for antibody production in animals. Further, the FGB1 and FGB2 polypeptides may include an amino acid residue, such as tyrosine or histidine, at the -COOH terminal end in order to facilitate the labeling of these novel polypeptides with a labeling moiety, such as radioactive iodine or phosphorous. When both the cysteine and tyrosine amino acid residues are added, the FGB1 and FGB2 polypeptides have calculated molecular weights of about 2,174 and 2,077, respectively. It is believed that the addition of the cysteine and tyrosine amino acid residues uniquely and advantageously enhances the reactivity of the immunogenic HCV polypeptides of the instant invention with antibodies in, for example, immuno assays. It is believed that the added cysteine and tyrosine amino acid residues may enhance the ability of the immunogenic HCV polypeptides to attach to the microwell plates possibly by interaction, especially between the tyrosine amino acid residues and such plates, in a manner that leaves the remainder or a greater portion of the immunogenic HCV polypeptides exposed to the reacting antibodies.

Examples of other novel basic HCV polypeptides in accordance with the present invention that are believed to be immunogenic and have epitopes for HCV are recited hereinafter. These polypeptides of the instant invention have been designated herein throughout as FGB polypeptides, and include:

FGB3-CRPQRKTKRNTNRRPQY; FGB4-CKPQRRTKRNTNRRPQY;

FGB5-CKPQRKTRRNTNRRPQY; FGB6-CKPQKKTKRNTNRRPQY;

FGB7-CKPQRKTKKNTNRPPQY; FGB8-CKPQRKTKRNTNKRPQY;

FGB9-CKPQRKTKRNTNRKPQY; FGB10-CKPQRKSKRNTNRRPQY;

FGB11-CKPQRKTKRNSNRRPQY; FGB12-CRKTKRNTNRRPQY;

FGB13-CKKTKRNTNRRPQY; FGB14-CRRTKRNTNRRPQY;

FGB15-CRKTRRNTNRRPQY; FGB16-CRKSKRNTNRRPQY;

FGB17-CRKTKKNTNRRPQY; FGB18-CRKTKRNTNKRPQY;

FGB19-CRKTKRNTNRKPQY; FGB20-CKQKKTKRSTNRRPQY;

FGB21-KQKKTKRSTNRRPQY; FGB22-KPQKKTKRSTNRRPQY;

FGB23-KPQKKNKRSTNRRPQY; FGB24-CKQRKTKRSTNRRPQ;

FGB25-KQRKTKRSTNRRPQY; FGB26-CKQRKTKRSTNRRPQY;

FGB27-KPQRKTKRSTNRRPQY; FGB28-CKQRKTKRNTNRRQY;

FGB29-KQRKTKRNTNRRQ; FGB30-KQRKTKRNTNRR;

FGB31-KPQRKNKRNTNRRPQ; FGB32-CKQRKTKRNTNRRPQY;

FGB33-KQRKTKRNTNRRPQ; FGB34-CKPQKKNKRNTNRRPQY;

FGB35-CKPQRKNKRNTNRRPQY; FGB36-KPQRKTKRNTNRRPQ;

FGB37-CKPQRKTKRNTNRRPQY; FGB38-KPQKKNKRNTNRRPQ.

In the above-recited amino acid sequences for the FGB3-FGB38 polypeptides, R or Arg represents argenine, N or Asn represents asparagine, C or Cys represents

cysteine, Q or Gln represents glutamine, K or Lys represents lysine, P or Pro represents proline, S or Ser represents serine, T or Thr represents threonine and Y or Tyr represents tyrosine.

The novel immunogenic HCV polypeptides, and especially the FGB1 and FGB2 polypeptides, may be used in diagnostic tests and kits, such as enzyme-linked immunosorbent assays (ELISA, EIA), radioimmunoassays (RIA), immunoblot assays, and the like, to detect the presence of specific HCV antibodies present in blood, sera, semen, or other biological materials of patients with HCV. For example, a coded HCV panel of 44 sera was tested in an ELISA using the FGB1 and FGB2 polypeptides. The FGB1 and FGB2 polypeptides utilized in this ELISA included the cysteine and tyrosine amino acid residues at their respective $-NH_2$ and -COOH terminal ends. The 44 sera were also tested for anti-C100 HCV antibody (EIA, Ortho/Chiron), recombinant immunoblot assay I (RIBA I, Ortho/Chiron), and neutralization (Neut., Abbott). Eight of eight samples that were reactive for C100 EIA, RIBA I and Neut., contained antibodies to FGB1 or FGB2 polypeptides. None of the six sera that were EIA reactive, but RIBA I indeterminate had antibodies to the FGB1 and FGB2 polypeptides. More significantly, however, two sera from a patient who had been clinically diagnosed as having non-A, non-B hepatitis

(NANBH) and were EIA and RIBA I non-reactive were reactive to the novel FGB1 and FGB2 polypeptide. One of the two such sera was reactive to the novel FGB2 polypeptide. These results are summarized in the HCV PANEL I and in TABLES 9 and 10 in greater detail.

HCV PANEL I

No. of Sera Samples	C100 EIA	RIBA I	Neut.	FGB1	FGB2
8	8r	8r	8y	8r	8r
6	6r	6i	3y	6nr	6nr
12	12r	12nr	6y	12nr	12nr
13	13nr	nt	nt	13nr	7nr
2*	2nr	2nr	nt	2r	1r
2	2r	nt	nt	2r	2nr
1	nt	nt	nt	1nr	1r

r-reactive; i-indeterminate; nr-non-reactive; nt-not tested; y-positive.

*clinically diagnosed NANBH patient, non-reactive with C100 EIA and RIBA I.

In a second coded HCV panel, 115 sera from patients with clinical evidence of NANB (type C) viral hepatitis or volunteer blood donors with or without evidence of NANB (type C) viral hepatitis were tested in an ELISA using the FGB1 polypeptide (HCV PANEL II). This panel was used to compare the ELISA utilizing FGB1 with an experimental C-200/C-22 ELISA (second generation of Chiron Corp.). Like with the first coded panel, the FGB1 polypeptide utilized in this ELISA included the cysteine and tyrosine amino acid residues at its respective $-NH_2$ and -COOH terminal ends. The 115 sera were also tested with C-100 ELISA (Ortho/Chiron Corp.) and recombinant immunoblot assay

II (RIBA II, Chiron Corp.) for HCV antibodies. All 115 sera were initially tested with the C-100 ELISA and all were reactive. Fifty-two sera (HCV PANEL II) that were reactive with C-200/C-22 ELISA and HCV-FGB1 ELISA were verified to be reactive with RIBA-II, with the exception of three sera that were indeterminate (one single band was reactive on RIBA-II). Forty sera non-reactive with C-200/C-22 ELISA and HCV-FGB1 ELISA were also found to be non-reactive with RIBA-II. Of two sera (HCV PANEL II, 2nr#) non-reactive with C-200/C-22 ELISA and HCV-FGB1 ELISA, one was reactive with RIBA-II and one was indeterminate. One serum sample (HCV PANEL II, 1r+) that was reactive with HCV-FGB1 ELISA was non-reactive with both the C-200/C-22 ELISA and RIBA-II. On the other hand, two serum sample (HCV PANEL II, 1nr°) that were reactive with both C-200/C-22 ELISA and RIBA-II tested negative with HCV-FGB1 ELISA. Both of these samples became reactive upon retesting with HCV-FGB1 ELISA. Finally, 18 sera which were reactive with C-200/C-22 ELISA tested negative with HCV-FGB1; 11 were negative with RIBA-II while 7 were indeterminate. While the majority (11/18 or 61%) appear to be false positive by the C-200/C-22 ELISA, the 7 (39%) that were indeterminate were reactive with one non-structural antigen, (C-100), of RIBA II, and therefore are most probably false positive by C-200/C-22 ELISA.

HCV PANEL II

Results of Comparative Testing of Coded Panel 2
(115 sera Reactive by the C-100 ELISA)

No. of Samples	HCV-SP ELISA	RIBA-II	HCV C-200 C-22 ELISA
52	52r	49r + 3i	52r
40	40nr	40nr	40nr
18	18nr	11nr + 7i	18r
2	2nr#	1r + 1i	2nr
1	1r+	1nr	1nr
2	2nr°	2r	2r
115			

r-reactive; nr-non-reactive; i-indeterminate (only one band out of four was reactive)

Sample	5-1-1	RIBA-II C-100	C33c	C22
#HCV055	-	1+	-	-
HCV203	1+	1+	-	-

+HCV225 HCV-SP ELISA (S/CO= 1.425)
o HCV214, HCV234 became reactive upon retesting with HCV-FGB1 ELISA.

In a third coded HCV panel, 1076 sera from volunteer, healthy, blood donors were also tested with the HCV-FGB1 ELISA (HCV PANEL III). As with the first two coded panels, the FGB1 polypeptide utilized in this ELISA included the cysteine and tyrosine amino acid residues at its respective $-NH_2$ and -COOH terminal ends. Eleven of the 1,076 samples reactive with HCV-FGB1 ELISA were further tested with RIBA-II and C-200/C-22 ELISA. One of the eleven sera was reactive by all three tests; 10 sera were repeat positives only by HCV-FGB1 ELISA. At this stage, it is unknown whether this reflects the rate of fals

positive tests (less than 1%) using HCV-FGB1 ELISA or whether the HCV-FGB1 ELISA can detect antibody to HCV undetected by the other two tests, i.e., C-200/C-22 ELISA and RIBA II.

HCV PANEL III

Results of Testing Health Blood Donors for Anti-HCV Antibodies

No. of DONORS	HCV-FGB1 REPEAT REACTIVE	RIBA-II	HCV C-200 C-22 ELISA
1076	11°	1*	1*

° Only the 11 samples identified reactive by HCV-FGB1 ELISA were tested by RIBA-II and the C-200/C22 ELISA.

* This sample was among those that were repeat reactive with HCV-FGB1 ELISA.

In a fourth coded HCV panel obtained from the Transfusion Transmitted Virus Study (TTVS) at the National, Heart, Lung and Blood Institute (NHLBI), Bethesda (MD, Hollinger, F.B. et al., 1982 in Viral Hepatitis: 1981 International Symposium, ed. Szmunes, W., Alter, H.J. and Maynard, J.E., Franklin Institute Press, Philadelphia, pp. 361-376), 94 sera obtained from serial bleeds were tested with the HCV-FGB1 ELISA, C-100 ELISA and RIBA II (HCV PANEL IV). These samples were selected from among those collected from blood transfusion recipients at 2 week intervals for 6 months, 3 week intervals for the next three months, and then at 10 months. A serum sample was also obtained, in most cases, on day 7 after the first

transfusion (recipients) or after surgery (controls). After reporting the results on this fourth coded panel, it was revealed that this panel consisted of 18 transfusion recipients and 10 control subjects (hospitalized, but not transfused). The transfusion recipients had been previously diagnosed as cases of acute or chronic hepatitis B and/or NANB hepatitis. Again, the FGB1 polypeptide utilized in this ELISA included the cysteine and tyrosine amino acid residues at its respective $-NH_2$ and -COOH terminal ends. The second generation C-200/C-22 ELISA was unavailable for this fourth coded panel. The results on each of these samples were matched to the different transfused patients or control subjects in this panel (HCV PANEL IV). Six control (non-transfused) subjects without evidence of hepatitis tested negative by all three assays. Four subjects (non-transfused) with a clinical diagnosis of NANB hepatitis tested negative by HCV-FGB1 ELISA; three of these four subjects also tested negative by the two other tests. Of the six transfusion recipients with clinical and laboratory diagnosed HBV hepatitis, the two also diagnosed with NANBH tested positive for HCV by all three tests. The remaining four recipients with HBV alone tested negative with HCV-FGB1 ELISA. Nine of nine recipients with diagnosed NANB hepatitis who tested positive with HCV-FGB1 ELISA were reactive on RIBA-II. In contrast,

three other recipients with NANB hepatitis tested negative by HCV-FGB1 ELISA, HCV C-100 ELISA; two of the three recipients were negative by RIBA-II, and one was indeterminate.

HCV PANEL IV

HCV-FGB1 ELISA, C-100 ELISA and RIBA-II Results in Recipients and Control Subjects from TTVS-NHLBI

	Total No. Subjects	FGB1 ELISA	C-100 ELISA	RIBA-II
Control^{+} - no NANB	6	NR	NR	NR
Control^{+} - NANB	3	NR	NR	NR
Control^{+} - NANB	1	NR	R	R
Recipient* - HBV	2	NR	NR	NR
	1	NR	R	NR
	1	NR	R	I
Recipient* - HBV and NANB	2	R	R	R
Recipient* - NANB (type HCV)	8	R	R	R
Recipient* - NANB (type HCV)	1	R	NR	R
Recipient* - NANB (non HCV)	2	NR	NR	NR
	1	NR	NR	I

+ Controls were hospitalized patients who did not receive a blood transfusion and were diagnosed as having or not having viral hepatitis.

* Recipients were patients who received one or more blood transfusions and were diagnosed as having viral hepatitis.

It should therefore now be apparent that the instant invention identifies new epitopes to the structural region of the human HCV genome to which HCV infected patients elicit antibodies. Based upon the results from the coded HCV panels, it is believed that these antibodies correlate with the presence of true positive HCV antibody as defined by C-100 EIA,

C-200/C22 EIA, RIBA I and RIBA II. Moreover, it is believed that assays using the truncated immunogenic HCV polypeptides and in particular the FGB1 and FGB2 polypeptides of the instant invention, which are derived from the structual region relatively near the predicted ATG initiation codon of the human HCV genome, to detect the presence of HCV are more specific and sensitive than assays using the recombinant C100 polypeptide, which is derived from the non-structual region of the HCV gene as well as the RIBA I or Neut. assays. Remarkably, the novel immunogenic HCV polypeptides of the instant invention are therefore believed to allow for significantly increased specificity in the detection of antibodies in blood or blood derived products contaminated with HCV or in patients with acute or chronic HCV at a relatively lower cost as compared to existing HCV tests.

The present invention also contemplates the detection of passive HCV-specific antibodies which have been transferred to patients via transfused blood containing detectable levels of the specific HCV antibodies. Quite remarkably, it is believed to now be possible to detect passive HCV-specific antibodies almost immediately following the initial transfusion initiated HCV infection when following the teachings of the present invention. Even more remarkable, it is

believed to now be possible to detect HCV-specific antibodies, i.e., passive HCV-specific antibodies followed by actively produced HCV-specific antibodies, in transfusion initiated HCV infected patients at relatively high levels for several months, as verified by the fifth coded panel and illustrated in FIGS. 11A-11G. Moreover, it is believed that the appearance of actively produced HCV-specific antibodies coincides with the first elevation of ALT levels. This is believed to have been confirmed using serial specimens from patients infected with transfusion associated NANB hepatitis. (Coded Panel 5). By "passive HCV-specific antibodies, it is meant herein to refer to those antibodies present in donated blood which are specific for HCV antigens and which have been produced by the donor. By "actively produced antibodies", it is meant herein to refer to those antibodies which are specific for HCV antigens but which have been produced by a person following HCV infection, such as a recipient of donated HCV-infected blood.

In order to document the time of appearance of HCV specific antibody responses in certain recipients from the above TTVS-NHLBI study who developed NANB hepatitis, we tested, using HCV-FGB1 ELISA, a coded panel of serial serum samples from six recipients and one control subject (Coded Panel 5) and correlated these results with the ALT levels. As with

the other coded panels, the FGB1 polypeptide utilized in this ELISA included the cysteine and tyrosine amino acid residues at its respective $-NH_2$ and -COOH terminal ends. HCV-specific antibody was detected by HCV-FGB1 ELISA in all six recipients and its appearance was found to coincide with the earliest elevation of ALT levels. Five out of the six recipients, with the exception of recipient #10805 (See FIG. 11D), on average had detectable HCV-specific active antibody within a month of transfusion transmitted HCV infection. (FIGS. 11B-11G). Significant levels of passive HCV-specific antibody, probably passively transferred from a donor, were detected during the first week post-transfusion for 3 recipients, i.e., recipient #s 30587, 21354 and 20621 (FIGS. 11F, 11C and G, respectively), with marginal presence in two other recipients, i.e., recipient #s 20959 and 10762 (FIGS. 11B and 11E, respectively). This same coded panel was tested subsequently under code with the C-200/C-22 ELISA. With the exception of a few test results, the data relative to the C-200/C-22 ELISA coincided with the HCV-SP specific antibody response, as reported in the description of FIGS. 11A-11G hereinafter. While FIGS. 11A-11G report the detection of passive HCV-specific antibody at seven days following transfusion, it is believed that passive HCV-specific antibody can be detected in a

recipient as early as about 20 minutes or earlier, e.g. whatever the time is required for blood to circulate, following transfusion with donated blood which contains detectable levels of passive HCV-specific antibody. It should be understood by those versed in this art that all five coded panels were tested with an HCV-FGB1 ELISA as described in Example I.

In another aspect of the present invention, it is concerned with the polynucleotide sequences which encode the two novel HCV polypeptides, FGB1 and FGB2, respectively, or degenerate polynucleotides which are complementary or correspond to all or certain nucleotides of the polynucleotide sequences. The present invention therefore contemplates the following novel polynucleotide sequences I and II which are believed to encode the novel FGB1 and FGB2 polyeptides, respectively:

(I)
5'-AAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAG-3';
(U) (U) (U)

and

(II)
5'-TCTCGGCCTAGTTGGGGCCCCACGGACCCCCGGCGTAGGTCGCGC-3';
(U U) (UU) (U) (U)

novel polynucleotides which are complementary or correspond to all or certain nucleotides thereof; and novel polynucleotide sequences obtained by substituting at least one nucleotide in each codon for

a corresponding amino acid of said polynucleotide sequences I and II, in accordance with degeneracy of the genetic code. The novel polynucleotide sequences of the instant invention may be inserted into recombinant expression vectors or the like and used to transform cells in order to recombinantly produce the FGB1 and FGB2 polypeptides. In another application of the novel polynucleotide sequences is their use in kits as probes or primers for the detection of RNA or nucleic acids from HCV in samples or other biological materials, such as sera, ascites, lymph, infected tissue, and cell cultures. The probes or primers which are complementary or correspond to the above-recited polynucleotide sequences should be of a sufficient length which allows for the detection of HCV RNA or DNA, respectively. For example, while 6-8 nucleotides complementary to or derived from the above-recited polynucleotide sequences may be a workable length, sequences of 10-12 nucleotides complementary thereto or derived therefrom are preferred and about 20-45 nucleotides and in particular about 20 nucleotides complementary or derived therefrom thereto appear to be most optimal.

The instant invention is also concerned with antisense single stranded DNA and RNA sequences derived from the polynucleotide sequences encoding the FGB1 or FGB2 polypeptides, which have the ability to

bind to HCV RNA and block protein translation and/or prevent HCV RNA replication. Still further, the present invention is concerned with ds DNA fragments which, when inserted into appropriate vectors such as retroviral vectors and introduced into infected liver cells results in the transcription of the antisense RNA sequences. As a further advantage, it is believed that such antisense polynucleotide sequences can be designed to include in their construct, for example, ribozymes that may play a role in the therapeutic treatment of HCV by catalytic degradation of the HCV RNA in the infected liver cells.

The present invention further contemplates the production of antibodies, polyclonal or monoclonal, raised against the novel immunogenic HCV polypeptides of the instant invention. In another aspect of the instant invention, it is concerned with the production of anti-idiotype antibodies raised against antibodies to the novel immunogenic HCV polypeptides. The novel antibodies of the present invention can be used in diagnostic tests and kits to detect HCV proteins (antigens) or anti-HCV antibodies, respectively. Moreover, it is believed that such antibodies of the present invention may have the unique ability to cross-react with host protein(s) to detect autoimmune liver disease induced by, for example, HCV.

Still another aspect of the instant invention is concerned with vaccines as prophylaxis against HCV infection by administering the novel immunogenic HCV polypeptides of the instant invention, either individually or in combination, to individuals. The vaccines of the instant invention comprise an immunogenic HCV polypeptide, such as FGB1 and/or FGB2 polypeptides, in pharmacologically effective doses mixed in pharmaceutically acceptable excipients. Preferably, the novel immunogenic HCV polypeptides of the instant invention are conjugated to, for example, carrier proteins, antibodies, or particle-forming proteins, such as those associated with hepatitis B surface antigen, and mixed with an adjuvant in order to elicit protective antibodies when administered to individuals. In addition, novel antibodies to the novel immunogenic HCV polypeptides of the present invention may be used for passive immunity in short term therapy against HCV.

Accordingly, it can now be appreciated that the present invention is believed to provide a solution to the HCV art that has long sought effective and inexpensive means to treat and reliably detect HCV, including HCV infection in acute and chronic phases.

The above features and advantages will be b tter understood with reference to the Figs.,

Examples and Detailed Description set out hereinbelow. It will also be understood that the biological materials of this invention are exemplary only and are not to be regarded as limitations of this invention.

Brief Description of the Figs.

Reference is now made to the accompanying Figs. in which are shown characteristics corresponding to the novel immunogenic HCV polypeptides of the instant invention from which certain of their novel features and advantages will be apparent:

Fig. 1 is a graphic illustration of the hydrophilicity and hydrophobicity (surface probability) profiles and the antigenic index of about the first 500 putative amino acids encoded by the structual gene of a human HCV isolate following the predicted ATG initiation codon;

Fig. 2 is a graphic illustration of the hydrophilicity and hydrophobicity (surface probability) profiles and the antigenic index of about the first 144 putative amino acids encoded by the structual gene of the human HCV isolate of Fig. 1 following the predicted ATG initiation codon;

Fig. 3 is a graphic illustration depicting that there are eight (8) amino acid residues of the Cys-FGB1-Tyr polypeptide present in its Chou-Fasman turn (C = cysteine, K = lysine, P = proline, Q =

glutamine, R = arginine, T = threonine, N = asparagine, Y = tyrosine);

Fig. 4 is a graphic illustration depicting that there are seven (7) amino acid residues of the FGB1 polypeptide present in its Chou-Fasman turn (K = lysine, P = proline, Q = glutamine, R = arginine, T = threonine, N = asparagine);

Fig. 5 is a graphic illustration depicting that there are seventeen (17) amino acid residues of the Cys-FGB2-Tyr polypeptide present in its Chou-Fasman turn (C = cysteine, S = serine, R = arginine, P = proline, W = tryptophan, G = glycine, T = threonine, D = aspartic acid, Y = tyrosine);

Fig. 6 is a graphic illustration depicting that there are twelve (12) amino acid residues of the FGB2 polypeptide present in its Chou-Fasman turn (S = serine, R = arginine, P = proline, W = tryptophan, G = glycine, T = threonine, D = aspartic acid);

Fig. 7 is a HPLC profile that shows the purity of the Cys-FGB1-Tyr polypeptide which has a retention time of about 12.45 minutes;

Fig. 8 is a HPLC profile that shows the purity of the Cys-FGB2-Tyr polypeptide which has a retention time of about 17.78 minutes;

Fig. 9 is a mass spectrum profile that shows the purity of the Cys-FGB1-Tyr polypeptide and the molecular weight thereof;

Fig. 10 is a mass spectrum profile that shows the purity of the Cys-FGB2-Tyr polypeptide and the molecular weight thereof; and

Figs. 11A-11G are correlations between antibodies to HCV-FGB1 (●——●) and ALT levels (——┼——) on serial samples from a control patient without hepatitis, i.e., recipient #10521 (Fig. 11A), and six transfusion recipients with NANB hepatitis. The number of units received by the recipients is indicated in parenthesis. The sample to cutoff (S/CO) ratio of 1.0 for the HCV-FGB1 ELISA is indicated by the horizontal line where values above the line indicate reactivity while those below the line are non-reactive. Results on the C-200/C-22 ELISA which differed from those of the HCV-FGB1 ELISA were as follows: All the serum samples of recipient #10805 (Fig. 11D) including the one withdrawn prior to receiving blood transfusion were found to be positive with C-200/C22 ELISA. The other differences were that, in recipient #s 21354 and 30587 (Figs. 11C and 11F, respectively), the passive HCV-specific antibodies, i.e., passively transferred HCV-specific antibodies from the donors, persisted for a longer time than the HCV-FGB1 specific antibodies, especially on days 21 and 17 respectively, and the serum sample withdrawn on day 29 for the recipient #21354 (Fig.

11C) was negative with C-200/C-22 ELISA, but positive with the HCV-FGB1 ELISA.

Detailed Description

By way of illustrating and providing a more complete appreciation of the present invention and many of the attendant advantages thereof, the following detailed description is provided concerning the novel immunogenic HCV polypeptides, polynucleotide sequences encoding the immunogenic HCV polypeptides and antisense polynucleotide sequences derived therefrom, antibodies raised against the immunogenic HCV polypeptides, anti-idiotype antibodies raised against the HCV antibodies to the immunogenic HCV polypeptides, HCV vaccines, and methods.

The present invention provides for the discovery of highly immunogenic HCV polypeptides, each having epitopes for HCV. The immunogenic HCV polypeptides of the instant invention have been derived from the putative amino acid sequence of the HCV polyprotein encoded by the five prime (5') end of a human isolate of a HCV genome. The amino acid residues of the epitopes of the immunogenic HCV polypeptides are believed to lie within the first 4% of the amino acid residues in the putative amino acid sequence of the HCV polyprotein.

The immunogenic HCV polypeptides of the instant invention are generally basic, truncated structural polypeptides of at least about 15 amino acids in length and are preferably between about 15 and about 17 amino acids. In addition, the immunogenic HCV polypeptides of the present invention have calculated molecular weights of at least about 1500 and preferably of between about 1500 and about 2500, and include at least about 7 amino acid residues (45%) in their Chou-Fasman (CF) turn, as evidenced by Figs. 3-6. As more particularly shown in Figs. 5 and 6, certain of the immunogenic HCV polypeptides of the instant invention may include at least about 12-17 amino acids residues (80%-100%) in their CF turns. See Chou, P.Y. and Fasman, G.D.: Biochemistry, 13:211-222 (1974); Chou, P.Y. and Fasman, G.D.: Biochemistry, 13:222-245 (1974); Chou, P.Y. and Fasman, G.D.: Ann. Rev. Biochem., 47:251-275 (1978); and Chou, P.Y. and Fasman, G.D.: Adv. Enzymol. Relat. Areas Mol. Biol., 47:45-148 (1978). Still further, at least about 11 of the amino acid residues (70%) within their amino acid sequences are hydrophillic. Preferably, however, between about 11 and 14 (70-87%) of the amino acid residues are hydrophillic. Still further, at least about 5 (30%) and preferably between about 5 and 8 (30-53%) of the amino acid residues of the immunogenic HCV polypeptides of the present invention

are polar amino acid residues. Moreover, the immunogenic HCV polypeptides of the instant invention have at least about 5 basic amino acid residues (30%) and more preferably between about 5 and 7 basic amino acid residues (30-47%). Accordingly, the estimated pI for the HCV polypeptides of the instant invention are typically on the order of about 12. In addition, the immunogenic HCV polypeptides have at least about 4 arginine amino acid residues (25%) in their amino acid sequences and more preferably include between about 4 and 7 arginine residues (25-47%).

In discovering the polypeptides of the instant invention, the first 500 or so putative amino acids following a predicted ATG initiation codon of a structural gene encoding a putative structural polyprotein derived from a human HCV isolate were subjected to hydrophilicity/hydrophobicity/antigenicity analysis. See Fig. 1. This was accomplished via a protein analysis program marketed under the name Mac Vector by International Bio Technologies Inc., P.O. Box 9558, New Haven, CT 06535. According to the hydrophilicity, surface probability and antigenic index plots as shown in Fig. 1, the first 144 or so putative amino acids following the predicted ATG initiation codon within the HCV structural gene encoding the putative structural polyprotein derived from the human HCV isolate presented what appears to

be a strong antigenic region. As a result, the hydrophilicity, surface probability and antigenic index plots of these first 144 putative amino acids were expanded, as shown in Fig. 2. The expanded index plots of the first 144 putative amino acids, as depicted in Fig. 2, revealed that these first 144 putative amino acids further presented several smaller regions which appear to be strongly antigenic. The results of this computer analysis provided the basis for the discovery of the novel highly immunogenic, basic truncated structural HCV polypeptides of the instant invention. It is understood by those of skill in the art, however, that such computer analysis of hydrophilicity, surface probability and antigenicity does not always identify epitopes that actually exist, and can also incorrectly identify regions of a putative amino acid sequence as containing epitopes.

In particular, the present invention provides for the discovery of two highly immunogenic, basic truncated structural HCV polypeptides, each having epitopes for HCV. These HCV polypeptides of the instant invention are designated herein throughout as FGB1 and FGB2 and, as indicated hereinbefore, have been derived from the putative amino acid sequence encoded by the five prime (5') end of a human isolate of a HCV genome. Moreover, their epitopes are believed to lie within the first 43 of amino acids

found in the putative amino acid sequence of the human HCV polyprotein. Still further, the amino acid sequences for FGB1 and FGB2 correspond to the putative amino acid sequences designated as 5-19 and 102-116, respectively, following the amino acid encoded by a predicted ATG initiation codon of a human HCV genome. The amino acid sequences for the novel FGB1 and FGB2 polypeptides, respectively are as follows:

SEQ ID NOS: 1-4

(Cys) - Lys - Pro - Gln - Arg - Lys - Thr - Lys - Arg - Asn - Thr - Asn - Arg - Arg - Pro - Gln -(Tyr); and

SEQ ID NOS: 5-8

(Cys) - Ser - Arg - Pro - Ser - Trp - Gly - Pro - Thr - Asp - Pro - Arg - Arg - Arg - Ser - Arg -(Tyr).

In the above-recited amino acid sequences, Arg (R) represents arginine, Asn (N) represents asparagine, Cys (C) respresents cysteine, Gln (Q) represents glutamine, Gly (G) represents glycine, Lys (K) represents lysine, Pro (P) represents proline, Ser (S) represents serine, Thr (T) represents threonine, Trp (W) represents tryptophan and Tyr (Y) represents tyrosine. The amino acids designated in parentheticals above represent optional but preferred amino acids added either individually or together to the immunogenic HCV polypeptide sequences for conjugation and labelling. Moreover, such optional amino acids and in particular the tyrosine amino acid

are belived to enhance the reactivity of the immunogenic HCV polypeptides of the instant invention by enhancing their attachment to, for example, the microwell plates possibly by interaction in such a manner that they leave the rest or greater portion of the novel HCV immunogenic polypeptides exposed to the reactive antibodies during, for example, an ELISA type assay. While Cys and Tyr represent preferred optional amino acids, other optional amino acid residues or other moieties suitable for the purposes stated herein are envisioned by the instant invention. For example, cystine may be substituted for the cysteine amino acid residue for conjugation purposes whereas histidine (His) may be substituted for the tyrosine amino acid residue for labeling purposes. Moreover, while the terms FGB1 and FGB2 as used herein refer broadly to the FGB1 and FGB2 polypeptides as either including or excluding one or both of the optional amino acids or moieties, respectively, when it is specifically intended for such polypeptides to include both of the optional amino acids, i.e., Cys and Tyr, they will be referred to herein as Cys-FGB1-Tyr and Cys-FGB2-Tyr, respectively.

When the FGB1 polypeptide includes the optional Cys and Tyr amino acid residues at its respective $-NH_2$ and -COOH terminal ends, it has 17 amino acids in its sequence, a molecular weight of

about 2174, a pI of about 12.484, 8 amino acids (53%) in its CF turn (Fig. 3), 14 hydrophillic amino acids (82%), 8 polar amino acids (53%), 7 basic amino acids (41%), 4 actual Arg amino acid residues (24%), and a combination of 4 Arg and 3 Lys amino acid residues (41%). On the other hand, when the FGB1 polypeptide is free of such optional, but preferred amino acids, it has 15 amino acids in its sequence, a molecular weight of about 1879, a pI of about 12.958, 7 amino acids (47%) in its CF turn (Fig. 4), 13 hydrophillic amino acid residues (87%), 7 polar amino acid residues (47%), 7 basic amino acid residues (47%), 4 actual Arg amino acid residues (27%), and a combination of 4 Arg and 3 Lys amino acid residues (47%). See Cys-FGB1-Tyr and FGB1 TABLES.

CYS-FGB1-TYR TABLE

Amino Acid Composition:

Non-polar:	Number	Percent
Ala	0	0.00
Val	0	0.00
Leu	0	0.00
Ile	0	0.00
Pro	2	11.76
Met	0	0.00
Phe	0	0.00
Trp	0	0.00
Polar:	**Number**	**Percent**
Gly	0	0.00
Ser	0	0.00
Thr	2	11.76
Cys	1	5.88
Tyr	1	5.88
Asn	2	11.76
Gln	2	11.76
Acidic:	**Number**	**Percent**
Asp	0	0.00
Glu	0	0.00
Basic:	**Number**	**Percent**
Lys	3	17.65
Arg	4	23.53
His	0	0.00

Calculated Molecular Weight = 2174.310
Estimated pI = 12.484

FGB1 TABLE
Amino Acid Composition:

Non-polar:	Number	Percent
Ala	0	0.00
Val	0	0.00
Leu	0	0.00
Ile	0	0.00
Pro	2	13.33
Met	0	0.00
Phe	0	0.00
Trp	0	0.00
Polar:	**Number**	**Percent**
Gly	0	0.00
Ser	0	0.00
Thr	2	13.33
Cys	0	0.00
Tyr	0	0.00
Asn	2	13.33
Gln	2	13.33
Acidic:	**Number**	**Percent**
Asp	0	0.00
Glu	0	0.00
Basic:	**Number**	**Percent**
Lys	3	20.00
Arg	4	26.67
His	0	0.00

Calculated Molecular Weight = 1908.010
Estimated pI = 12.958

With respect to the FGB2 polypeptide, when it includes the optional, but preferred amino acid residues, i.e., the Cys and Tyr amino acid residues at its respective $-NH_2$ and -COOH terminal ends, it has 17 amino acids, a molecular weight of about 2077, a pI of about 12.306, 17 amino acids (100%) in its CF turn (Fig. 5), 12 hydrophillic amino acid residues (71%), 7 polar amino acid residues (41%), 5 basic amino acid residues (29%), 5 actual Arg amino acid residues (29%), and no Lys amino acid residues. However, when the FGB2 polypeptide is free of the optional Cys and Tyr amino acid residues at its respective $-NH_2$ and -COOH termini, it has 15 amino acids, a molecular weight of about 1811, a pI of about 12.656, 12 amino acids (80%) in its CF turn (Fig. 6), 11 hydrophillic amino acid residues (73%), 5 polar amino acid residues (33%), 5 basic amino acid residues (33%), 5 actual Arg amino acid residues (33%), and no Lys amino acid residues. See Cys-FGB2-Tyr and FGB2 TABLES.

CYS-FGB2-TYR TABLE

Amino Acid Composition

Non-polar:	Number	Percent
Ala	0	0.00
Val	0	0.00
Leu	0	0.00
Ile	0	0.00
Pro	3	17.65
Met	0	0.00
Phe	0	0.00
Trp	1	5.88
Polar:	**Number**	**Percent**
Gly	1	5.88
Ser	3	17.65
Thr	1	5.88
Cys	1	5.88
Tyr	1	5.88
Asn	0	0.00
Gln	0	0.00
Acidic:	**Number**	**Percent**
Asp	1	5.88
Glu	0	0.00
Basic:	**Number**	**Percent**
Lys	0	0.00
Arg	5	29.41
His	0	0.00

Calculated Molecular Weight = 2077.210
Estimated pI = 12.306

FGB2 TABLE

Amino Acid Composition:

Non-polar:	Number	Percent
Ala	0	0.00
Val	0	0.00
Leu	0	0.00
Ile	0	0.00
Pro	3	20.00
Met	0	0.00
Phe	0	0.00
Trp	1	6.67
Polar:	**Number**	**Percent**
Gly	1	6.67
Ser	3	20.00
Thr	1	6.67
Cys	0	0.00
Tyr	0	0.00
Asn	0	0.00
Gln	0	0.00
Acidic:	**Number**	**Percent**
Asp	1	6.67
Glu	0	0.00
Basic:	**Number**	**Percent**
Lys	0	0.00
Arg	5	33.33
His	0	0.00

Calculated Molecular Weight = 1810.910
Estimated pI = 12.656

Other examples of novel FGB HCV polypeptides in accordance with the present invention that are believed to be immunogenic and have epitopes for HCV are recited hereinafter. It should be understood that while the FGB1 and FGB2 polypeptides as well as those FGB polypeptides having, inter alia, at least about 15 amino acid residues, at least about 7 amino acid residues in their Chou-Fasman Turn and at least about 4 arginine amino acid residues are presently preferred, the present invention contemplates other equivalent polypeptides whose fingerprint characteristics may fall somewhat outside of the parameters set forth herein to define the basic, immunogenic HCV polypeptides.

FGB TABLE

	CMW	EpI	AA	CFT	R	HP	P	B	K
FGB3	2202.36	12.658	17	8	5	15	8	7	2
FGB4	2202.36	12.658	17	8	5	15	8	7	2
FGB5	2202.36	12.658	17	10	5	15	8	7	2
FGB6	2146.26	12.195	17	8	3	15	8	7	4
FGB7	2146.26	12.195	17	8	3	15	8	7	4
FGB8	2146.26	12.195	17	8	3	15	8	7	4
FGB9	2146.26	12.195	17	5	3	15	8	7	4
FGB10	2160.28	12.484	17	11	4	15	8	7	3
FGB11	2160.28	12.484	17	10	4	15	8	7	3
FGB12	1820.94	12.484	14	8	4	13	7	6	2
FGB13	1792.89	12.192	14	8	3	13	7	6	3
FGB14	1848.99	12.658	14	13	5	13	7	6	1
FGB15	1848.99	12.658	14	10	5	13	7	6	1
FGB16	1806.91	12.484	14	14	4	13	7	6	2
FGB17	1792.89	12.192	14	8	3	13	7	6	3
FGB18	1792.89	12.192	14	8	3	13	7	6	3
FGB19	1792.89	12.192	14	5	3	13	7	6	3
FGB20	2022.12	12.195	16	11	3	15	8	7	4
FGB21	1918.99	12.195	15	11	3	14	7	7	4
FGB22	2016.1	12.195	16	11	3	14	7	7	4
FGB23	2029.1	12.195	16	13	3	14	7	7	4
FGB24	1887	12.958	15	10	4	14	7	7	3
FGB25	1947.04	12.484	15	11	4	14	7	7	3
FGB26	2050.17	12.484	16	11	4	15	8	7	3
FGB27	2044.15	12.484	16	11	4	14	7	7	3
FGB28	1980.09	12.484	15	7	4	15	8	7	3
FGB29	1713.79	12.958	13	6	4	13	6	7	3
FGB30	1585.66	12.958	12	5	4	12	5	7	3
FGB31	1921.01	12.958	15	12	4	13	6	7	3
FGB32	2077.2	12.484	16	8	4	15	8	7	3
FGB33	1810.9	12.958	14	7	4	13	6	7	3
FGB34	2159.26	12.195	17	13	3	15	8	7	4
FGB35	2187.31	12.484	17	13	4	15	8	7	3
FGB36	1908.01	12.958	15	7	4	13	6	7	3
FGB37	2174.31	12.484	17	8	4	15	8	7	3
FGB38	1892.96	12.784	15	12	3	13	6	7	4

CMW = Calculated Molecular Weight
EpI = Estimated pI
AA = Amino Acids
CFT = Chou-Fasman Turn
R = Arginine
HP = Hydrophilic Amino Acids
P = Polar Amino Acids
B = Basic Amino Acids
K = Lysine

Examples of other FGB polypeptides include,

FGB3-CRPQRKTKRNTNRRPQY; FGB4-CKPQRRTKRNTNRRPQY;

FGB5-CKPQRKTRRNTNRRPQY; FGB6-CKPQKKTKRNTNRRPQY;

FGB7-CKPQRKTKKNTNRPPQY; FGB8-CKPQRKTKRNTNKRPQY;

FGB9-CKPQRKTKRNTNRKPQY; FGB10-CKPQRKSKRNTNRRPQY;

FGB11-CKPQRKTKRNSNRRPQY; FGB12-CRKTKRNTNRRPQY;

FGB13-CKKTKRNTNRRPQY; FGB14-CRRTKRNTNRRPQY;

FGB15-CRKTRRNTNRRPQY; FGB16-CRKSKRNTNRRPQY;

FGB17-CRKTKKNTNRRPQY; FGB18-CRKTKRNTNKRPQY;

FGB19-CRKTKRNTNRKPQY; FGB20-CKQKKTKRSTNRRPQY;

FGB21-KQKKTKRSTNRRPQY; FGB22-KPQKKTKRSTNRRPQY;

FGB23-KPQKKNKRSTNRRPQY; FGB24-CKQRKTKRSTNRRPQ;

FGB25-KQRKTKRSTNRRPQY; FGB26-CKQRKTKRSTNRRPQY;

FGB27-KPQRKTKRSTNRRPQY; FGB28-CKQRKTKRNTNRRQY;

FGB29-KQRKTKRNTNRRQ; FGB30-KQRKTKRNTNRR;

FGB31-KPQRKNKRNTNRRPQ; FGB32-CKQRKTKRNTNRRPQY;

FGB33-KQRKTKRNTNRRPQ; FGB34-CKPQKKNKRNTNRRPQY;

FGB35-CKPQRKNKRNTNRRPQY; FGB36-KPQRKTKRNTNRRPQ;

FGB37-CKPQRKTKRNTNRRPQY; FGB38-KPQKKNKRNTNRRPQ.

It should be further understood that while the sequences to certain of the above FGB polypeptides include the cysteine and/or tyrosine amino acid residue at their respective $-NH_2$ and -COOH terminal ends, any such FGB polypeptide or immunogenic HCV polypeptide of the present invention may be altered to delete or include only the cysteine or tyrosine amino acid residue or both such residues at their respective

terminal ends in accordance with the teachings of the instant invention.

The immunogenic HCV polypeptides and in particular the FGB1 and FGB2 polypeptides of the instant invention can be produced by chemical snythesis, recombinant technology or any other methods available in the art so long as the methodology selected does not interfere with their utilities stated herein. Moreover, alternative amino acid residues may be substituted for those recited in the above sequences for the FGB1 and FGB2 polypeptides so long as the substituted amino acids do not defeat the utility of such novel immunogenic HCV polypeptides. For example, it is believed that Lys and Arg amino acid residues may be freely substituted for each other within the amino acid sequences. It is likewise believed that Ser and Thr amino acid residues may be freely substituted for one another. Thus, it is to be understood that the reference herein to at least about 4 Arg amino acid residues (25%) means that the sequences of the immunogenic HCV polypeptides of the instant invention include at least about 4 Arg amino acid residues or at least about 4 Lys amino acid residues or a combination of at least about 4 Lys and Arg amino acid residues. For example, the above-recited sequence for the FGB1 polypeptide has about 7 Arg amino acid residues (41%), i.e., 4 Arg and 3 Lys,

when the optional amino acids are included and about 7 Arg amino acid residues (47%), i.e., 4 Arg and 3 Lys, when the optional amino acids are excluded. Preferably, however, it means that the sequences for the immunogenic HCV polypeptides of the instant invention include at least about 4 actual Arg amino acid residues (25%) and more preferably about 4 actual Arg amino acid residues and up to about 3 actual Lys amino acid residues (up to about 47%). Likewise, selected amino acids may be added to or deleted from the amino acid sequences of the HCV immunogenic polypeptides of the instant invention to expand or shorten same so long as the objectives of the instant invention are not defeated. For instance, as already indicated hereinbefore, Cys and Tyr amino acid residues can be added to the respective amino and carboxyl termini of the FGB1 and FGB2 polypeptides without interfering with their function as HCV epitopes. Thus, such modified or varied amino acid sequences are considered to be equivalents to the immunogenic HCV polypeptides and in particular the FGB1 and FGB2 polypeptides and are contemplated within the scope of the present invention.

As previously stated, the immunogenic HCV polypeptides and in particular the FGB1 and FGB2 polypeptides of the instant invention can be synthetically produced. For example, the Cys-FGB1-Tyr

and Cys-FGB2-Tyr polypeptides can be synthesized on an Applied BioSystem Peptide Synthesizer 430 using solid phase strategy, as described in Merrifield, F.B.: J. Am. Chem. Soc., 85:2149-2154 (1963) and Mitchell, A. R. et al.: J. Org. Chem., 43:2845-2854 (1978). Boc amino acids (t-Boc) are activated as benzotriazole (HOBt) esters and coupling is performed in N-methyl-pyrrolidinone. The side chain protecting groups are Asp (OBzl), Ser (OBzl), Thr (OBzl), Lys (Cl-z), Arg (Tos), Tyr (Br-z), Trp (CHO), Cys (p-MeBzl). Syntheses can be performed starting with 0.5 mmole of BocTyr (BrZ) Pam resin. Deprotection and cleavage of the Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides from the resin can be performed using hydrogen fluoride. See Tables 2, 3, 4 and 5. The Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides can be purified to greater than 95% using reversed-phase HPLC. Analytical HPLC using the applied Biosystems 130A separation system with a microbore column show the Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides to be greater than 98% pure. See Figs. 7 and 8. The authenticity of the Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides can be confirmed by amino acid analysis using Applied Biosystems 420A automated hydrolyzer, see Tables 6 and 7, and by molecular weight determination using the Bio-Ion mass analyzer. See Figs. 9 and 10. It should be understood that the Cys-FGB1-Tyr and Cys-FGB2-Tyr

polypeptides referenced in TABLES 1-7, Figs. 3, 5, 7-10 and Examples I-III specifically include the optional Cys amino acid residues at the amino termini and Tyr amino acid residues at the carboxyl termini.

TABLE 2

Cys-FGB1-Tyr Polypeptide

CYCLE NUMBER	AMINO ACID	REACTION VESSEL	CONCENTRATOR VESSEL	TEMP	ACTIVATOR VESSEL
	BEG	RBEG11 R	CBEG 11	10c	ABEG11
1	Gln	RBOC 11R	CBOC 11	10c	ABOC 12
2	Pro	RBOC 11R	CBOC 11	10c	ABOC 11
3	Arg	RBOC 11R	CBOC 11	10c	ABOC 14
4	Arg	RBOC11DR	CBOC 11D	10c	ABOC 14D
5	Asn	RBOC11DR	CBOC 11D	10c	ABOC 13D
6	Thr	RBOC 11R	CBOC 11	10c	ABOC 11
7	Asn	RBOC11DR	CBOC 11D	10c	ABOC 13D
8	Arg	RBOC 11R	CBOC 11	10c	ABOC 14
9	Lys	RBOC11DR	CBOC 11D	10c	ABOC 13D
10	Thr	RBOC11DR	CBOC 11D	10c	ABOC 11D
11	Lys	RBOC 11R	CBOC 11	10c	ABOC 13
12	Arg	RBOC11DR	CBOC 11D	10c	ABOC 14D
13	Gln	RBOC 11R	CBOC 11	10c	ABOC 12
14	Pro	RBOC11DR	CBOC 11D	10c	ABOC 11D
15	Lys	RBOC 11R	CBOC 11	10c	ABOC 13
16	Cys	RBOC 11R	CBOC 11	10c	ABOC 11
	END	RNH211 R	CEND 11	10c	AEND11

TABLE 3

Cys-FGB1-Tyr POLYPEPTIDE

RES. NUMBER	AMINO ACID	BOC MW. OF ACID	TOTAL WEIGHT OF PEPTIDE RESIN	SUBSTITUTION (mmol/g)	COMMENT
				0.630	INITIAL SUB
17	Tyr	000	00.796	0.630	
16	Gln	246	00.860	0.581	
15	Pro	215	00.908	0.550	
14	Arg	429	01.064	0.470	(TOS)
13	Arg	429	01.219	0.410	(TOS)
12	Asn	232	01.276	0.392	
11	Thr	309	01.372	0.364	(Bzl)
10	Asn	232	01.429	0.350	
09	Arg	429	01.584	0.316	(TOS)
08	Lys	415	01.733	0.289	(Cl-Z)
07	Thr	309	01.828	0.274	(Bzl)
06	Lys	415	01.977	0.253	(Cl-Z)
05	Arg	429	02.132	0.235	(TOS)
04	Gln	246	02.196	0.228	
03	Pro	215	02.245	0.223	
02	Lys	415	02.393	0.209	(Cl-Z)
01	Cys	325	02.497	0.200	(4-MeBzl)

TABLE 4

Cys-FGB2-Tyr POLYPEPTIDE

CYCLE NUMBER	AMINO ACID	REACTION VESSEL	CONCENTRATOR VESSEL	TEMP	ACTIVATOR VESSEL
	BEG	RBEG11 R	CBEG 11	10c	ABEG11
1	Arg	RBOC 11R	CBOC 11	10c	ABOC 14
2	Ser	RBOC 11R	CBOC 11	10c	ABOC 11
3	Arg	RBOC 11R	CBOC 11	10c	ABOC 14
4	Arg	RBOC11DR	CBOC 11D	10c	ABOC 14D
5	Arg	RBOC11DR	CBOC 11D	10c	ABOC 14D
6	Pro	RBOC11DR	CBOC 11D	10c	ABOC 11D
7	Asp	RBOC 11R	CBOC 11	10c	ABOC 11
8	Thr	RBOC11DR	CBOC 11D	10c	ABOC 11D
9	Pro	RBOC11DR	CBOC 11D	10c	ABOC 11D
10	Gly	RBOC 11R	CBOC 11	10c	ABOC 11
11	Trp	RBOC 11R	CBOC 11	10c	ABOC 13
12	Ser	RBOC 11R	CBOC 11	10c	ABOC 11
13	Pro	RBOC11DR	CBOC 11D	10c	ABOC 11D
14	Arg	RBOC11DR	CBOC 11D	10c	ABOC 14D
15	Ser	RBOC 11R	CBOC 11	10c	ABOC 11
16	Cys	RBOC 11R	CBOC 11	10c	ABOC 11
	END	RNH211 R	CEND 11	10c	AEND11

TABLE 5

Cys-FGB2-Tyr POLYPEPTIDE

RES. NUMBER	AMINO ACID	BOC MW. OF ACID	TOTAL WEIGHT OF PEPTIDE RESIN	SUBSTITUTION (mmol/g)	COMMENT
				0.630	INITIAL SUB
17	Tyr	000	00.796	0.630	
16	Arg	429	00.951	0.525	(TOS)
15	Ser	295	01.040	0.481	(Bzl)
14	Arg	429	01.195	0.418	(TOS)
13	Arg	429	01.351	0.370	(TOS)
12	Arg	429	01.506	0.332	(TOS)
11	Pro	215	01.555	0.322	
10	Asp	323	01.657	0.302	(OBzl)
09	Thr	309	01.753	0.285	(Bzl)
08	Pro	215	01.801	0.278	
07	Gly	175	01.830	0.273	
06	Trp	332	01.937	0.258	CHO
05	Ser	295	02.026	0.247	(Bzl)
04	Pro	215	02.074	0.241	
03	Arg	429	02.230	0.224	(TOS)
02	Ser	295	02.318	0.216	(Bzl)
01	Cys	325	02.422	0.206	(4-MeBzl)

TABLE 6

AMINO ACID ANALYSIS OF Cys-FGB1-Tyr

	NANOMOLES	THEORETICAL	ACTUAL
Asp (D)	0.47	2.00	2.05
Glu (E)	0.45	2.00	1.90
Ser (S)	0.00	0.00	0.00
Gly (G)	0.00	0.00	0.00
His (H)	0.00	0.00	0.00
Arg (R)	0.89	4.00	3.90
Thr (T)	0.45	2.00	1.90
Ala (A)	0.00	0.00	0.00
Pro (P)	0.49	2.00	2.10
Tyr (Y)	0.24	1.00	1.00
Val (V)	0.00	0.00	0.00
Met (M)	0.00	0.00	0.00
Cys (C)*	0.00	0.00	0.00
Ile (I)	0.00	0.00	0.00
Leu (L)	0.00	0.00	0.00
Phe (F)	0.00	0.00	0.00
Trp (W)	0.00	0.00	0.00
Lys (K)	0.67	3.00	2.90
Ave.	0.23		

Molecular weight: 2,174.5

*Cys amino acid residues are undetectable by an Applied Biosystems 420 A automated hydrolyzer.

TABLE 7

AMINO ACID ANALYSIS of Cys-FGB2-Tyr

	NANOMOLES	THEORETICAL	ACTUAL
Asp (D)	0.23	1.00	0.90
Glu (E)	0.00	0.00	0.00
Ser (S)	0.67	3.00	2.80
Gly (G)	0.24	1.00	1.00
His (H)	0.00	0.00	0.00
Arg (R)	1.23	5.00	5.20
Thr (T)	0.23	1.00	0.90
Ala (A)	0.00	0.00	0.00
Pro (P)	0.73	3.00	3.10
Tyr (Y)	0.23	1.00	0.90
Val (V)	0.00	0.00	0.00
Met (M)	0.00	0.00	0.00
Cys (C)*	0.00	0.00	0.00
Ile (I)	0.00	0.00	0.00
Leu (L)	0.00	0.00	0.00
Phe (F)	0.00	0.00	0.00
Trp (W)	0.00	0.00	0.00
Lys (K)	0.00	0.00	0.00
Ave.	0.24		

Molecular weight: 2,077.32

*Cys amino acid residues are undetectable by an Applied Biosystems 420A automated hydrolizer.

The HCV polypeptides including the FGB1 and FGB2 polypeptides of the present invention also can be produced by recombinant techniques well known to those of skill in the art. That is, they can be produced by gene expression of a ds DNA comprising at least one of the polynucleotide sequences which encode the polypeptides and especially the FGB1 and/or the FGB2 polypeptides of the instant invention, or a degenerate polynucleotide sequence obtained therefrom by, for example, substituting at least one nucleotide in one of the polynucleotide sequences encoding the FGB1 or FGB2 polypeptides in accordance with degeneracy of genetic code. As recited hereinbefore, a polynucleotide sequence encoding the FGB1 polypeptide is:

SEQ ID NOS: 9-16

```
5'-(TGT) AAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCC
   (TGC)      (U)                (U)          (U)
   (UGU)
   (UGC)
         ACAG (TAT)-3'
              (TAC)
              (UAU)
              (UAC)
```

whereas a polynucleotide sequence encoding the FGB2 polypeptide is:

SEQ ID NOS: 17-24

```
5'-(TGT) TCTCGGCCTAGTTGGGGCCCCAGGGACCCCCGGCGTAGGTC
   (TGC)(U U)   (U)(UU)                       (U) (U)
   (UGU)
   (UGC)

         GCGC (TAT)-3'.
              (TAC)
              (UAU)
              (UAC)
```

In the above-mentioned polynucleotide sequences and herein throughout, when in the form of DNA, A represents a deoxyriboadenylic acid residue (dAMP), G represents a deoxyriboguanylic acid residue (dGMP), C represents a deoxyribocytidylic acid residue (dCMP), and T represents a thymidylic acid residue (TMP), and when in the form of RNA, A represents a riboadenylic acid residue (AMP), G represents a riboguanylic acid residue (GMP), C represents a ribocytidylic acid (CMP), and U, which is substituted for T, represents a ribouridylic acid residue (UMP). The left and right ends of each sequence recited in the above-mentioned polynucleotide sequences represent the 5'-hydroxyl group side and 3'-hydroxyl group side, respectively. It of course should be understood that in the event the immunogenic HCV polypeptides including the FGB1 and FGB2 polypeptides are to be recombinantly expressed with the Cys and Tyr amino acid residues, the appropriate codons must be included in the expressing polynucleotide sequences, and such polynucleotide sequences are within the contemplation of the instant invention. Such codons which encode for Cys and Tyr amino acid residues are well known to those of skill in the art and include, for example, TGT or TGC (DNA) or UGU or UGC (RNA) for Cys and TAT or TAC (DNA) or UAU or UAC (RNA) for Tyr. The substitution or addition of a nucleotide of either of the

above-mentioned oligonucleotide sequences can be performed by the methods of, for example, Saiki, et al.: Science, 230:1350-1354 (1985), and Saiki, et al.: Science, 239:487-491 (1988).

Using the polynucleotide sequences disclosed herein, a DNA library can be prepared by customary methods. That is, the polynucleotide sequences can be individually ligated to replicable cloning vectors to thereby obtain DNA libraries. As a replicable cloning vector, any known or commercially available vectors, such as phage genes, cosmids, plasmids and animal virus genes may be used. When a phage gene or a cosmid is used as a replicable vector, in order to attain high stability and high transforming ability of the vector after the DNA sequences have been individually inserted therein, the in-vitro packaging of each of the polynucleotide sequences inserted vectors is conducted by a customary method. Thus, the DNA-inserted vectors are obtained in the form of recombinant phage particles. The obtained phage particles are used as a DNA library for DNA cloning. On the other hand, when a plasmid is used as a replicable vector, the above-mentioned polynucleotide sequences, either individually or in combination, can be inserted in the plasmid vectors and the resultant DNA-inserted vectors are then individually introduced into host cells, such as cells of Escherichia coli, Bacillus

subtili, yeast or the like, according to a customary method. The thus obtained transformants are used as a DNA library for DNA cloning. Further, when an animal virus gene is used as a replicable vector, any of the above-mentioned polynucleotide sequences may be either individually or in combination inserted in the virus gene vectors and the resultant recombinant viruses are then individually transfected into animal cells according to a standard method and multiplied in the cells. In the case of a recombinant virus, the obtained recombinant viruses as such can be used as a DNA library.

When the DNA library is comprised of transformants, the transformants can be cultured on a standard agar medium to form colonies. On the other hand, when the DNA libraries are comprised of recombinant phage particles or recombinant viruses, these phage particles or recombinant viruses can be used to infect known host cells, such as Escherichia coli, Bacillus subtilli, yeast, animal cell culture and the like, and culture to form a plaque, or to multiply the infected cells.

The polynculeotides of the instant invention may be further used to recombinantly express the novel immunogenic HCV polypeptides including the FGB1 and FGB2 polypeptides or variations thereof, respectively. In order to recombinantly express the polynucleotide

sequences encoding the FGB1 and FGB2 polypeptides especially on a commercial scale, the polynucleotide sequences encoding the FGB1 and FGB2 polypeptides, whether in fused or free form, and whether or not containing a signal sequence to permit secretion, can be ligated into a replicable expression vector by a customary method which is suitable to any convenient host. Both eukaryotic and prokaryotic host systems can be used in forming the recombinant FGB1 and FGB2 polypeptides or variations thereof. The polynucleotide sequences can be synthetically produced or derived from clones. When a polynucleotide sequence of the instant invention is inserted into an expression vector to form a recombinant which is introduced in a compatible host, an individual polypeptide can be produced by gene expression. When such a polynucleotide sequence in combination with a DNA fragment is utilized to form a recombinant, a polypeptide can be expressed in the form of a fused or free polypeptide comprising peptides encoded by the inserted polynucleotide sequence and DNA fragment. A recombinant which is capable of expressing a polypeptide of the instant invention includes a coding sequence comprised of a polynucleotide sequence of the instant invention which is operably linked to a control sequence that is compatible with a desired host. In this form, such a

recombinant may be simply referred to herein as a recombinant expression system.

By the term "control sequence," it is meant herein to refer a to polynucleotide sequence which effects expression of a coding sequence to which it is ligated. The nature of such a control sequence may differ depending upon the host organism. For example, in prokaryotes, such control sequences generally include promoters, ribosomal binding sites, and terminators. In eukaryotes, however, such control sequences generally include promoters, terminators and, in some instances, enhancers. The term "control sequence" is therefore intended to include, at a minimum, all components whose presence is necessary for expression, and may also include additional components whose presence is advantageous, such as, leader sequences. By the term "operably linked", it refers herein to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequence. The term "coding sequence" as used herein refers to a polynucleotide sequence which may be transcribed into RNA and/or translated into a polypeptide when placed under the control of

appropriate regulatory sequences. The boundaries of the coding sequence are determined by an initiation codon at the 5'-terminus and a termination codon at the 3'-terminus. A coding sequence may include, but is not limited to RNA, DNA, and recombinant polynucleotide sequences. By the term "open reading frame", it is meant herein to refer to a region of a polynucleotide sequence which encodes a peptide or polypeptide. This region may represent a portion of a coding sequence or a total coding sequence.

As a replicable expression vector which may be used, any conventionally known or commercially available expression vector can be used. Examples of expression vectors include plasmid vector pSN508 for enterobacteria, U.S. Patent No. 4,703,005, plasmid vector pBH103 for yeast, and its series, Japanese Patent Application Laid-Open Specification No. 63-22098, plasmid pJM105, Japanese Patent Application Laid-Open Specification No. 62-286930, an attenuated chickenpox virus gene, Japanese Patent Application Laid-Open Specification No. 53-41202, an attenuated Marek's disease virus, J. Jap. Soc. Vet., 27:20-24 (1974) and Gan Monograph on Cancer Res., 10:91-107 (1971), plasmid pSC11, Chakrabarti et al.: Mol. Cell. Biol., 5:3403-3409 (1985), plasmid pUV1, Falkner et al.: Nucleic Acid. Res., 15:7192 (1987), plasmid pTM3, Moss et al.: Nature, 348:91-92 (1990), plasmid

pTTQ series which is sold by Amersham International, England, plasmid pSIV series which is sold by Pharmacia LKB, Sweden, plasmid pGEM^{TM}-3ZF(-) sold by Promego, Madison, WI, and the like, depending on the level of expression desired. The expression vectors containing inserted immunogenic HCV polynucleotides of the instant invention can be individually introduced or transfected into host cells sensitive to the vector according to conventional methods, to obtain transformants. By culturing the transformants or recombinant viruses obtained above according to customary methods, the FGB1 and FGB2 polypeptides can be produced in the culture of the transformants or the recombinant viruses and purified therefrom in accordance with standard techniques on a commercial scale.

The FGB1 and FGB2 polypeptides recombinantly produced may be isolated from lysed cells or from the culture medium of the transformants or recombinant viruses and purified to the extent needed for their intended use. Purification may be by any appropriate combination of customary techniques known in the art and selected from, for example, salt fractionation; adsorption and desorption using biogel; differential extraction; precipitation by an inorganic solvent; fractionation by ultracentrifugation; chromatography on ion exchange resins; affinity chromatography; high-performance liquid chromatography;

electrophoresis, and the like. When the FGB1 and FGB2 polypeptides are purified from the culture of an E. coli transformant or a yeast transformant, from a viewpoint of effective removal of allergens derived from E. coli and yeast which cause the quality of the final product of the FGB1 and FGB2 polypeptides to be markedly lowered, it is preferred that the purification be conducted by, for example, the steps of adsorption and elution using a biogel, such as a sepharose or agarose biogel. When the FGB1 and FGB2 polypeptides are purified from the culture of a recombinant virus, e.g., the culture of recombinant virus-infected cells, high purity FGB1 or FGB2 polypeptide can be obtained by subjecting a crude solution containing the polypeptide to purification by ultracentrifugation and density gradient centrifugation repeatly. Thus, a solution containing purified FGB1 or FGB2 polypeptide of the present invention is obtained. If desired, the solution may be lyophilized to obtain purified FGB1 or FGB2 polypeptide in a dry form.

Such recombinantly expressed FGB1 and FGB2 polypeptides can be used as diagnostics or formulated into vaccines. Antibodies raised against these recombinantly expressed polypeptides can also be used as diagnostics, or for passive immunotherapy. In addition, antibodies to these recombinantly expressed

polypeptides or anti-idictype antibodies, which are raised against such antibodies, are useful for isolating and identifying HCV.

The polynucleotide sequences of the present invention encoding the FGB1 and FGB2 polypeptides may also be used for diagnosing HCV hepatitis by nucleic acid and in situ hybridization technique. More particularly, the polynucleotide sequences described herein permits the construction of probes which are useful for detecting HCV RNA in biological samples, including blood, lymph, ascites, hepatocytes, etc. By the term "probe," it is used herein to refer to a polynucleotide which forms a hybrid structure with a sequence in a target region, due to complementarity of at least one sequence in the probe with a sequence in the target region. As used herein, the term "target region" refers to a region of nucleic acids which is to be amplified and/or detected.

For example, it is possible to prepare RNA or DNA oligomers of approximately 6 nucleotides or more which are complementary to the disclosed RNA polynucleotide sequences, either by excision or synthetically, which hybridize with the HCV genome and are useful in identifcation of HCV RNA in, for example, donated blood, sera of subjects suspected of harboring the virus, or cell culture systems in which the viruses replicate. The probes for HCV should be

of a length which allows the detection of HCV RNA or DNA sequences by hybridization. While 6-8 nucleotides complementary or corresponding to the disclosed polynucleotide sequences may be of a workable length, sequences of 10-12 nucleotides complementary or corresponding thereto are prefered and about 20-45 nucleotides and in particular 20 nucleotides complementary or corresponding thereto appear optimal.

The probes of the instant invention can be prepared using routine methods, including automated oligonucleotide synthetic methods, such as described in Warner: DNA, 3:401 (1984), which is incorporated herein by reference in its entirety. Among useful probes, for example, are those derived from the polynucleotide sequences, the novel clones disclosed herein, as well as the various oligomers useful in the DNA libraries, set forth herein. It should be appreciated that when the nucleotide sequences of the instant invention are used as probes, complete complementarity is desirable, although it may be unnecessary as the length of the probe is increased as indicated hereinbefore.

For use of such probes as diagnostics, the biological sample to be analyzed, such as blood, serum ascites, lymph and hepatocytes, may be processed, if desired, to extract HCV nucleic acids contained therein. The resulting nucleic acids from the sample

may be subjected to gel electrophoresis or other size separation techniques; alternatively, the nucleic acid sample may be dot blotted without size separation. The probes of the instant invention can then be labeled with a labeling moiety. For example, the probes may be labeled with ^{32}P by treatment with polynucleotide kinase in the presence of gamma ^{32}P-ATP, using standard conditions for the reaction, or by nick translation. Suitable other labels are known in the art, and include, for example, other radioactive labels such as radioactive iodine, biotin, alkaline phosphatase, fluorescent probes and chemiluminescent probes. The labeling of the probes may be performed by standard techniques known in the art such as by using commercially available nick translation kits or multiprime kits, such as those distributed by Amersham International, England and Nippon Gene Co., Ltd., Japan. The nucleic acids extracted from the sample can then be treated with the labeled probe under hybridization conditions of suitable stringencies, and polynucleotide duplexes containing the probe are detected.

The RNA and DNA probes of the instant invention are believed to be highly complementary to the HCV RNA genome and cDNA, respectively. Therefore, usually high stringency conditions are not required during hybridization. Nevertheless, to ensure against

false positives, conditions of high stringency should be used. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, length of time, and concentration of formamide. These factors are outlined in, for example, Sambrook, J. et al.: Molecular Cloning. A Laboratory Manual, 2nd., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989).

In carrying out such an assay, the labeled probe is introduced, for example, in a plastic bag. The amount of the labeled probe placed in the plastic bag is generally between about 1 and 100 nanograms per bag. The labeled probe may be contained in the plastic bag in the form of a solution. The diagnosis of HCV hepatitis by the use of the labeled probe is conducted by a standard hybridization method. That is, total RNA extracted from plasma, serum leukocytes, ascites fluid, lymph, or hepatocytes obtained from a patient is first electrophoresed and blotted or simply blotted on nitrocellulose. The blots of HCV RNA are then hybridized with the labeled probes in the bag, and the hybridized labeled probes are detected by, for example, autoradiogram in the positive HCV samples.

The probes can be packaged into diagnostic kits. Diagnostic kits include the DNA or RNA probes,

which may be labeled; alternately, the probes of the instant invention may be unlabeled and the ingredients for labeling may be included in the kit in separate containers. The kit may also contain other suitably packaged reagents and materials needed for the particular hybridization protocol, for example, standards, as well as instructions for conducting the test.

The polynucleotides of the instant invention may be further used to prepare anti-HCV viral agents, such as antisense polynucleotide fragments, which may interfere with HCV RNA replication, transcription or translation. Antisense polynucleotide fragments as used herein constitute single stranded DNA or RNA fragments derived from the polynucleotide sequences encoding the FGB1 or FGB2 polypeptides, e.g., complementary thereto, which permits them to bind specifically to designated regions of HCV RNAs. Antisense polynucleotide fragments of the instant invention include, for example, single stranded DNA or RNA fragments that have the ability to bind to HCV RNA to block protein translation and/or prevent replication of HCV RNA by polymerase. In addition, the instant invention contemplates double stranded (ds) DNA fragments which, when ligated into an appropriate expression vectors such as a retroviral vector and introduced into infected liver cells, transcribe complementary antisense RNA fragments that will bind

to HCV RNA to block protein translation and/or HCV replication.

As indicated hereinbefore, antisense single stranded polynucleotides which bind to HCV RNA according to the present invention are designed based upon the polynucleotide sequences encoding the FGB1 or FGB2 polypeptides provided herein. The antisense polynucleotides of the instant invention may be further designed to include, for instance, ribozymes which catalyze with high specificity, the degradation of HCV RNA. Hence, they may be delivered in specialized systems, for example, liposomes or by retroviral vectors used in gene therapy. In addition, they may include bonding between modified bases, analogs, etc. The antisense single stranded polynucleotide fragments of the present invention may also include molecules which carry agents (non-covalently attached or covalently bound) for enhancing their stability and effectiveness to degrade HCV RNA. Still further, the antisense polynucleotides of the instant invention may also bind to cellular RNA which enhance and/or are required for viral infectivity, replicative ability, or chronicity.

Examples of antisense single stranded RNA or DNA fragments contemplated by the instant invention include:

SEQ ID NOS: 25-26

UUUGGAGUUUCUUUUUGGUUUGCAUUGUGGUUGGCAGCGGGUGUC; and
(TTT) (TTT TTTTT)(TTT) (TT T)(TT) (T T)

SEQ ID NOS: 27-28

AGAGCCGGAUCAACCCCGGGGUCCCUGGGGGCCGCAUCCAGCGCG.
(T) (T) (T) (T)

Examples of ds DNA fragments, which when ligated in frame into appropriate vectors such as retroviral vectors and introduced into infected liver cells, transcribe the above antisense polynucleotide RNA sequences, include:

SEQ ID NO: 29

(I) 5′-GACACCCGCTGCCAACCACAATGCAAACCAAAAAGAAAC
3′-CTGTGGGCGACGGTTGGTGTTACGTTTGGTTTTTCTTTG

TCCAAA-3′
AGGTTT-5′; and

SEQ ID NO: 30

(II) 5′-CGCGCTGGATGCGGCCCCCAGGGACCCCGGGGTTGA.
3′-GCGCGACCTACGCCGGGGGTCCCTGGGGCCCCAACT

TCCGGCTCT-3′
AGGCCGAGA-5′.

By the term "in frame" as used herein throughout, it refers to the proper positioning of a desired sequence of nucleotides within a DNA fragment or coding sequence operably linked to a control sequence that results in optimal transcription or translation.

It of course should be understood that the above antisense single stranded DNA and RNA fragments and ds DNA fragments transcribing the antisense single

stranded RNA fragments can be shortened, expanded or modified as indicated hereinbefore so long as such modifications do not interfere with the ability of the antisense fragments to block HCV protein translation and/or HCV replication.

The immunogenic HCV polypeptides in accordance with the present invention can be prepared as discrete polypeptides or incorporated into larger polypeptides. For example, the FGB1 or FGB2 polypeptides can be prepared as unfused polypeptides or fused polypeptides in combination with one another, or the FGB1 and/or FGB2 polypeptides can be prepared in combination with other polypeptides to form heterologous fusion polypeptides. Useful heterologous polypeptide sequences include polypeptide sequences that provide for secretion from a recombinant host, enhance the immunological reacitivity of the HCV epitope(s), facilitate the coupling of the polypeptides to immunoassay supports or vaccine carriers, or ennance the solubility of the polypeptides. See, e.g., EPO Pub. No. 116,201; U.S. Pat. No. 4,722,840; EPO Pub. No. 259,149; U.S. Pat. No. 4,629,783. The above-referenced immunogenic HCV polypeptides and especially the FGB1 and FGB2 polypeptides of the instant invention therefore can be prepared as discrete or combination polypeptides or incorporated into larger polypeptides, and may find use as described herein.

Further, the HCV polypeptides and in particular the FGB1 and FGB2 polypeptides of the present invention may advantageously be used as active ingredients in vaccines for HCV. Vaccine preparations, which contain immunogenic polypeptides as active ingredients, are well known to those of skill in the art. Typically, such vaccines are injectibles, either as liquid solutions or suspensions; solid forms suitable for solution or suspension in liquid prior to injection may also be prepared. The preparation may also be emulsified, or the FGB1 or FGB2 polypeptides encapsulated in liposomes. The active immunogenic FGB1 and/or FGB2 polypeptides can be mixed with excipients which are pharmaceutically acceptable and compatible with the active ingredient. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol, or the like and combinations thereof. If desired, the vaccines may also contain minor amounts of auxillary substances such as wetting or emulsifying agents, pH buffering agents, and/or adjuvants which enhance the effectiveness of the vaccine. For example, to a solution containing a FGB1 and/or FGB2 polypeptide concentration of about 1 to about 500 micrograms per ml, aluminum hydroxide gel is added as an adjuvant so that the concentration of the added gel becomes about 0.1 to about 1.0 mg per ml. As an adjuvant, there may also be employed

precipitating depositary adjuvants such as calcuim phosphate gel, aluminum phosphate gel, aluminum sulfate, alumina hydroxide, alumina and bentonite. Examples of still further adjuvants include but are not limited to: N-acetyl-muramyl-L-threonine-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, i.e., monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL + TDM + CWS) in a 2% squalene/Tween 80 emulsion. The effectiveness of an adjuvant may be determined by measuring the amount of antibodies directed against the FGB1 or FGB2 polypeptide resulting from the administration of any of these polypeptides and vaccines in combination with various adjuvants.

In the event that the immunogenic HCV polypeptides and in particular the FGB1 and FGB2 polypeptides of the instant invention are too small to encompass the total immunogenicity of the structural region of the putative HCV structural protein, they may be linked to a suitable carrier. A number of techniques for obtaining such linkage are known in the

art, including the formation of disulfide linkages using N-succinimidyl-3-(2-pyridylthio)propionate(SPDP) and succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate(SMCC) available through Pierce Company, Rockford, IL. Since the FGB1 and FGB2 polypeptides lack a sulfhydryl group, this can be accomplished by providing, for example, a Cys amino acid residue or a cystine moiety on one protein (e.g., the FGB1 or FGB2 polypeptide) and an amide linkage through an epsilon-amino on a lysine or other free amino group in the other (e.g., carrier). A variety of such disulfide/amide-forming agents are known. See, for example, Harlow and Lane: Antibodies. A Laboratory Manual, pp. 130-131, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988). Other bifunctional coupling agents form a thioether rather than a disulfide linkage. Many of the thio-ether-forming agents are commercially available and include reactive esters of 6-maleimidocapric acid, 2-bromoacetic acid, 2-iodacetic acid, 4-(N-maleimidomethyl)cyclohexane-1-carboxylic acid, and the like. The carboxyl groups can be activated by combining them with succinimide or 1-hydroxyl-2-nitro-4-sulfonic acid, sodium salt. Additional methods of coupling antigens employs the rotavirus/"binding peptide" system described in, for example, EPO Pub. No. 259,149. The foregoing list is not meant to be exhaustive, and modifications of the

recited compounds can clearly be used. In addition to cysteine, it should be understood that any other amino acid residue or moiety, such as cystine, may be used that is suitable for this purpose so long as the objectives of the instant invention are not defeated.

It should also be understood that any carrier may be used which preferably does not itself induce the production of antibodies harmful to the host. Suitable carriers are typically large, slowly metabolized macromolecules such as polypeptides, polysaccharides, such as latex functionalized sepharose, agarose, cellulose, cellulose beads and the like; polymeric amino acids, such as polyglutamic acid, polylysine, and the like; amino acid copolymers; and inactive virus particles. Especially useful polypeptides substrates are serum albumins, keyhole limpet hemocyanin protein (KLH), immunoglobulin molecules, thyroglobulin, ovalbumin, tetanus toxoid, and other proteins well known to those versed in the art.

The vaccines can be conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; said suppositories may be

formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 1%-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, cellulose, magnesium carbonate, glucose, fructose, galactose, sucrose, lactose, albumin, gelatin, and amino acids such as glycine, alanine, lysine, arginine, glutamine, and the like. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained released formulations or powders and may contain, for example, 10%-95% of active ingredient, and preferably 25%-70%.

The FGB1 and FGB2 polypeptides may be formulated into a vaccine as neutral or salt forms. Pharmaceutically acceptable salts include the acid addition salts (formed with free amino groups of the peptide) and which are formed with inorganic acid, such as, for example, hydrochloric or phosphoric acids, or such organic acids such as acetic, oxalic, tartaric, maleic, and the like. Salts formed with the free carboxyl groups may also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, and ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, 2-ethylamino ethanol, histidine, procaine, and the like.

A vaccine of the instant invention may also be prepared in the form of a mixed vaccine which contains adsorbed HCV polypeptides, preferably adsorbed FGB1 and/or FGB2 polypeptides, mentioned above and at least one antigen other than the present FGB1 and/or FGB2 polypeptide. As the antigen other than the present FGB1 and/or FGB2 polypeptides, there may be employed any antigens that are conventionally used as active ingredients of the corresponding vaccines in so far as the side effects and adverse reactions caused by such other antigens and the FGB1 and/or FGB2 polypeptides are not additively or synergistically increased by the use of the FGB1 and/or FGB2 polypeptides and such other antigens in combination, and the antigenicities and immunogenicities of the FGB1 and/or FGB2 polypeptides and such other antigens are not reduced by the interference between the FGB1 and/or FGB2 polypeptides and other antigens. The number and the types of the antigens which may be mixed with the FGB1 and/or FGB2 polypeptides are not limited in so far as the side effects and adverse reactions are not increased additively or synergistically and the antigenicity and immunogenicity of each of the FGB1 and FGB2 polypeptides and such antigens are not reduced as mentioned above. Generally, two to six or more types of antigens may be mixed with the FGB1 and/or FGB2 polypeptides. Examples of

antigens which may possibly be mixed with the present FGB1 and/or FGB2 polypeptides, include detoxified antigens, inactivated antigens or toxoids which are derived from encephalitis virus, hfrs virus (hemorrhagic fever with renal syndrome), influenza virus, parainfluenza virus, HAV, HBV, dengue fever virus, AIDS virus, Bordetella pertussis, Diptheria bacillus, Tetanus bacillus, meningococcus, pneumococcus, and the like.

The immunogencity of the HCV polypeptides including the FGB1 and FGB2 polypeptides may also be enhanced by preparing them in mammalian or yeast systems fused with or assembled with particle-forming proteins such as, for example, that associated with hepatitis B surface antigen. Constructs wherein, for example, the FGB1 and/or FGB2 polypeptides are linked directly to the particle-forming protein coding sequences produce hybrids which are HCV immunogenic. In addition, the vectors may be prepared so that they include epitopes specific to HBV, having various degrees of immunogencity, such as, for example, the pre-S peptide. Thus, particles constructed from particle-forming proteins which include for instance the FGB1 and/or FGB2 polypeptides are immunogenic with respect to HCV and HBV.

Hepatitis surface antigen (HBSAg) has been shown to be formed and assembled into particles in S.

cerevisiae, Valenzuela, P. et al.: Nature, 298:344 (1982), as well as in, for example, mammalian cells Valenzuela, P. et al.: IN: Hepatitis B, Millman et al., ed, Plenum Press, pp. 225-236 (1984). The formation of such particles has been shown to enhance the immunogencity of the monomer subunit. The constructs may also include the immunodominant epitope of HBSAg, comprising the 55 amino acids of the presurface (pre-S) region. Neurath et al.: Science, 224:392 (1984). Constructs of the pre-S-HBSAg particle expressible in yeast are disclosed in EPO Pub. No. 174,444 whereas hybrids including heterologous viral sequences for yeast expression are disclosed in EPO Pub. No. 175,261. These constructs may also be expressed in mammalian cells such as Chinese hamster ovary (CHO) cells using an SV40-dihydrofolate reductase vector disclosed in Michelle, M.T. et al.: Viral Hepatitis and Liver Disease, Ed. Vyas, G.N., Dienstag, J.L. and Hoofnagle J.H., Abstract No. 8A.16, p. 659, Grune & Stratten, Inc., (1984).

Generally, a vaccine comprising, for example, the FGB1 and/or FGB2 polypeptides of the present invention may be contained and sealed in a vial, an ampule or the like. The vaccines of the present invention may be administered in the form of a liquid or suspension. In the case where the vaccine is in a dried form, the vaccine is disolved or suspended in

sterilized-distilled water or normal saline before administration, the amount of distilled water or normal saline being such that the volume becomes the original volume before being subjected to lyophilization. The vaccines should be administered in a manner compatible with the dosage formulation, and in such an amount as will be prophylactically and/or therapeutically effective. The quantity to be administered, which is believed to be generally in the amount of about 5 micrograms to about 250 micrograms or more of FGB1 or FGB2 per dose, depends on the subject to be treated, capacity of the subject's immune system to synthesize antibodies, and the degree of protection desired. Precise amounts of active ingredient required to be administered may depend on the judgement of the practitioner and may be peculiar to each subject. The dose of the vaccine per child may be half as much as that of the vaccine per adult. The vaccine may be given in a single dose schedule, or preferably in a multiple dose schedule. A multiple dose schedule is one in which a primary dose of vaccination may be given in 1-10 separate doses, followed with other doses given at subsequent time intervals required to maintain and/or boost the immune response, for example, at 1-4 months for a second dose, and if needed, a subsequent dose(s) after several months. The dosage regimen will also, at

least in part, be determined by the need of the individual, and be dependent upon the judgement of the practitioner.

In addition, the vaccine containing the novel immunogenic HCV polypeptides such as the FGB1 and FGB2 polypeptides may be administered in conjunction with other immunoregulatory agents, such as, immune globulins, interleukins, etc.

The novel immunogenic HCV polypeptides and especially the FGB1 and FGB2 polypeptides may also be used for preparing an antibody, such as a polyclonal antibody and a monoclonal antibody, specific for the immunogenic HCV polypeptides. For example, a polyclonal antibody specific for a KLH coupled-Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide may be prepared by conventional methods as follows. The KLH coupled- Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide of the present invention is innoculated subcutaneously, intramuscularly, intraperitoneally or intravenously into an animal such as mouse, guinea pig, rabbit, goat, horse, etc. The innoculation of the FGB1 or FGB2 polypeptide is preferably conducted several times at intervals of 1 to 4 weeks, to thereby completely immunize the animal. In order to enhance the immunizing effect, a conventionally commercially available adjuvant may be used as described hereinbefore. Serum from the immunized animal is collected

and the polyclonal antibody is isolated and purified from the blood serum according to standard techniques well known in the art, such as described in Mayer and Walker, eds. Immunochemical Methods in Cell and Molecular Biology, Academic Press, London (1987).

Monoclonal antibodies specific for the FGB1 or FGB2 polypeptides may also be prepared by conventional techniques as described in, for example, Harlow, E. and Lane, D.: Antibodies. A Laboratory Manual, pp. 139-244, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988), and by ImmunoZAP™ technology as reported in Antibody Expression Library Technology, distributed by Stratacyte, 11099 N. Torey Pines Rd., Suite 400, La Jolla, CA 92037. See also Marx, J.: Science, 246:1250-1251 (Dec. 8, 1989); and Huse, W.D. et al.: Science, 246:1275-1281 (Dec. 8, 1989). For example, when using conventional techniques, the splenic cells obtained from a mouse immunized with FGB1 and/or FGB2 polypeptide are fused with commercially available mouse myeloma cells by cell fusion technique, to obtain hybridomas. The hybridomas are screened to obtain a hybridoma capable of producing an antibody reactive with either the FGB1 or the FGB2 polypeptide. The obtained hybridoma is cultured in accordance with standard methods. From the supernatant of the culture, a monoclonal antibody

against either the FGB1 or the FGB2 polypeptide is isolated and purified by standard techniques.

The above-mentioned polyclonal and monoclonal antibodies may also be used as diagnostic agents for diagnosing HCV, and those which are neutralizing are useful in passive immunotherapy. The diagnosis of HCV using the antibody may be conducted by immunoassay in substantially the same manner as mentioned above with respect to the diagnosis of HCV using the FGB1 or FGB2 polypeptides. By the use of the polyclonal or the monoclonal antibodies, the identification and quantification of HCV antigen peptides present in, for example, a liver tissue, blood, sera, semen, lymph and ascites can be determined.

In addition, the novel antibodies and in particular the novel polyclonal antibodies raised against the immunogenic HCV polypeptides, especially the FGB1 polypeptide, of the instant invention may be useful for detecting autoimmune liver disease induced by, for example, HCV. In other words, it is believed that the immunogenic HCV polypeptides and in particular the FGB1 polypeptide of the present invention share epitopes with host protein(s) possibly expressed or released by the liver in response to inflammation, trauma or disease. Thus, the immunogenic HCV polypeptides and in particular the FGB1 polypeptide of the

instant invention are therefore believed to be effective for raising antibodies and in particular polyclonal antibodies capable of cross-reacting with HCV epitopes as well as other epitopes shared between HCV antigen(s) and host protein(s) for detecting autoimmune liver disease in immuno-type assays such as an immunofluorescence assay known to those of skill in the art.

Recently, it has been reported that an amino acid sequence Gly-Arg-Arg-Gly-Gln-Lys-Ala-Lys-Ser-Asn-Pro-Asn-Arg-Pro-Leu (GOR epitope), derived from the sequence of a clone which was isolated from the plasma of a laboratory chimpanzee infected with human parenteral NANBH (HCV), detected what was believed to be an autoantibody believed to have been generated by patients in response to acute or chronic parenteral NANBH (HCV) infection. See Mishiro, S. et al.: The Lancet, 336:1400-1403 (Dec. 8, 1990) and The Lancet, 336:1414-1415 (Dec. 8, 1990). With respect to autoimmune disease, it was also reported that if dissimilar amino acid(s) in the epitopes shared between the host protein and the foreign antigen constitute a radical substitution so as to interfere with antibody binding, virtual homology between the two may not lead to a cross-reacting immune response. See Oldstone, M.B.A.: Cell, 50:819-820 (Sept. 11, 1987). For

example, Oldstone reports that a high degree of homology between epitopes of the acetylcholine receptor alpha chain (Pro-Glu-Ser-Asp-Gln-Pro-Asp-Leu) and polyoma virus middle T antigen (Pro-Glu-Ser-Asp-Gln-Asp-Gln-Leu) fails to cause cross-reacting antibodies, whereas a more distant similarity between the acetylcholine receptor sequence and herpes simplex virus glycoprotein D (Pro-Asn-Ala-Thr-Gln-Pro-Glu-Leu) induces strong immunological cross-reacitivity. The underscored amino acids represent similar amino acids. See Oldstone, M.B.A.: Cell, 50:819-820 (Sept. 11, 1987) and Dyrberg and Oldstone: Curr. Topics Microbiol. Immunol., 130:25-37 (1986).

With respect to the novel FGB1 polypeptide of the instant invention, it has a distant similarity to the recently reported GOR epitope by Mishiro, S. et al. from the plasma of a laboratory chimpanzee infected with human parenteral NANBH (HCV). As already indicated herein, the GOR epitope has a reported sequence

Gly-Arg-Arg-Gly-Gln-Lys-Ala-lys-Ser-Asn-Pro-Asn-Arg-Pro-Leu,

whereas the sequence for the novel FGB1 polypeptide is Lys-Pro-Gln-Arg-Lys-Thr-Lys-Arg-Asn-Thr-Asn-Arg-Arg-Pro-Gln. The underscored amino acids represent

distant, but similar amino acids between the two epitopes.

Monclonal antibodies, in particular, may be used to raise anti-idiotype antibodies. Anti-idiotype antibodies are immunoglobulins which carry an "internal image" of the antigen of the infectious agent against which protection is desired. See, for example, Nisonoff, A. et al.: Clin. Immunol. Immunopathol., 21:397-406 (1981) and Dreesman et al.: J. Infect. Disease, 151:761 (1985). Techniques for raising anti-idiotype antibodies are well known in the art. See, for example, Grych et al.: Nature, 316:74 (1985), MacNamara et al.: Science, 226:1325 (1984), and Vytdehaag et al.: J. Immunol., 134:1225 (1985). The anti-idiotype antibodies generated may also be useful for treatment and/or diagnosis of HCV by, for example, reacting with HCV antigen or detecting the presence of anti-HCV antibody in biological samples.

It should also be recoginized by one of ordinary skill in the art that a variety of types of antibodies directed against the immunogenic HCV polypeptides including the FGB1 or FGB2 polypeptides may be produced. As used herein, the term "antibody" refers to a polypeptide or group of polypeptides which are comprised of at least one antibody combining site. An "antibody combining site" or "binding domain" is formed from the folding of variable domains of an

antibody molecule(s) to form three-dimensional binding spaces with an internal surface shape and charge distribution complementary to the features of an epitope of an antigen, which allows an immunological reaction with the antigen. An antibody combining site may be formed with a heavy and/or a light chain domain (VH and VL, respectively), which form hypervariable loops which contribute to antigen binding. The term "antibody" therefore is used broadly herein and includes, for example, vertebrate antibodies, hybrid antibodies, chimeric antibodies, ImmunoZAP™ antibodies, altered antibodies, univalent antibodies, the Fab proteins, and single domain antibodies, as known in the art.

Both the FGB1 and FGB2 polypeptides and the antibodies raised against such polypeptides are useful in immunoassays to detect presence of HCV antibodies, or the presence of HCV and/or HCV antigens, in biological samples, such as blood, sera and semen. Design of the immunoassays is subject to a great deal of variation, and a variety of these are well known in the art. For example, the immunoassay may utilize one HCV epitope; alternatively, the immunoassay may use a combination of HCV epitopes derived from for instance the FGB1 and FGB2 polypeptides. It may use, for example, a monclonal antibody directed towards either the FGB1 or FGB2 polypeptide, a combination of

monoclonal antibodies directed towards the FGB1 and FGB2 polypeptides, polyclonal antibodies directed towards either the FGB1 or FGB2 polypeptides, or a combination of polyclonal antibodies directed toward the FGB1 and FGB2 polypeptides. Protocols may be based, for example, upon competition, direct reaction, or sandwich type assays. Protocols may also, for example, use solid supports, or may be by immunoprecipitation.

Preferably, the assays of the present invention involve the use of antibodies or polypeptides and in particular the FGB1 and/or FGB2 polypeptides of the instant invention labeled with a labeling moiety. It is to be understood that the term "labeling moiety" is used herein throughout in a broad sense and is meant to include, but not limited to, radioisotopes, enzymes, antibodies, dyes, chemiluminescent or fluorescent molecules, immune complexes, and the like, which may be directly or indirectly attached to the antibody, polypeptide or polynucleotide sequence of the instant invention. In order to radioactively label the FGB1 or FGB2 polypeptides, it is preferable to conjugate a Tyr or His amino acid residue or the like to the carboxyl end thereof as discussed hereinbefore. Techniques for radioactively labeling antibodies and polypeptides are well known to those of skill in the art and may be

utilized to label the antibodies and immunogenic HCV polypeptides of the instant invention. For example, the techniques disclosed in Harlow and Lane: Antibodies. A Laboratory Manual, Chapter 9, pp 324-339, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988), and Weir, D.M.: Radioimmunoassay, Chapter 17, (IN):Immunochemistry, Vol. 1, 2nd ed., Blackwell Scientific Publications, London (1973) may be employed to label the antibodies and immunogenic HCV polypeptides of the present invention with radioiodine. Examples of immuno assays which may be utilized include enzyme-labeled and radioactive immunoassays, such as ELISA and RIA assays, respectively.

Kits suitable for immunodiagnosis and containing the appropriate labeled reagents are constructed by packaging the appropriate materials, including the novel immunogenic HCV polypeptides such as the FGB1 and/or FGB2 polypeptides of the invention or antibodies raised against such immunogenic HCV polypeptides or anti-idiotype antibodies raised against antibodies to such immunogenic HCV polypeptides in suitable containers, along with the remaining reagents and materials required to conduct the assay, as well as a suitable set of assay instructions.

It is to be understood by those versed in this art that the practice of the present invention

employs, unless otherwise indicated herein, conventional techniques of molecular biology, biochemistry, microbiology, virology, recombinant DNA, and immunology, which are within the skill of the art. All patents, patent applications, and publications cited herein, both supra and infra, are hereby incorporated herein by reference in their entireties.

Examples of various embodiments of the present invention will now be further illustrated with reference to the following Examples.

Example I

Enzyme-Linked Immunosorbent Assay (ELISA) for Detection of HCV Antibodies and Diagnostic Kit

Polypeptides FGB1 and FGB2 are used as antigens in an ELISA diagnostic assay as described below to detect antibodies to HCV in biological samples.

Materials and Reagents

1. Phosphate Buffered Saline (PBS) (SIGMA, P.O. Box 14508, St. Louis, Mo. 63178, catalog no. D6650)
2. 96 well microtiter plates (Dynatech Immunolon U shaped wells. Dynatech Laboratories, Inc., 900 Slaters Lane, Alexandria, Va 22314).
3. Parafilm (or other cover for plates)
4. Tween 20 (Biorad Laboratories, 1414 Harbour Way South, Richmond, Ca 94804)
5. Rabbit anti-human IgG conjugated to horse radish peroxidase (Dako Corporation, 6392 Via Real, Carpinteria, Ca 93013)
6. OPD (1,2-phenylenedianine, dihydrochloride) tablets (2mg/tablet) (Dako Corporation, 6392 Via Real, Carpinteria, Ca 93013)
7. 0.1 M Citric Acid-Phosphate Buffer pH 5.0
   -Citric Acid*H_2
   -Na_2HPO_4*$12H_2O$
8. 30% Hydrogen Peroxide (H_2O_2)
9. Plastic Forceps or Tweezers
10. Aluminum Foil
11. 1 M Sulfuric Acid (H_2SO_4)

PBS:Tween (Wash Solution)

PBS containing 0.1% Tween 20

Citric Acid-Phosphate Buffer

Dissolve 7.3g Citric Acid and 23.88g Na_2HPO_4 in 1 L H_2O. Store at 4°C.

Substrate Solution

Bring OPD tablets and Citric Acid-Phosphate Buffer to room temperature. Use plastic forceps or tweezers to retrieve OPD tablets. For every 96 well microtiter plate, dissolve 4 OPD tablets in 12 mls of 0.1 M Citric Acid-Phosphate Buffer pH 5.0. Add 5 uls 30% H_2O_2. The solution is maintained at room temperature and used within one hour of preparation. Since it is light sensitive, the container is kept wrapped in aluminum foil until used.

Procedure

Dissolve Cys-FGB1-Tyr and/or Cys-FGB2-Tyr polypeptide in PBS at a concentration of about 1mg/ml. Dilute the polypeptide solution to 1:100 in PBS (10 ng of polypeptide). Add 100uls of diluted polypeptide solution to each well to be used. Cover plate with parafilm and incubate for about 8-12 hours on a shaker platform at room temperature. Remove polypeptide solution and wash wells by adding 200 uls PBS:Tween to each, cover plate and incubate on shaker for 4-6 hours at room temperature. Remove wash solution and add 80uls PBS:Tween. Add 20 uls of patient or normal serum to each well. Cover plate and incubate overnight on a shaker platform at room temperature.

Remove samples and wash 3 times with 100 uls PBS:Tween. Dilute rabbit anti-human IgG conjugated to horse radish peroxidae 1:200 in PBS:Tween. Add 100 uls diluted rabbit anti-human IgG to each well. Cover plate and incubate for about 3-6 hours on a shaker platform at room temperature. Remove rabbit anti-human IgG and wash 3 times with 100 ul PBS:Tween. Add 100 uls Substrate Solution to each well. Cover plate and incubate for about 5 min. at room temperature. Add 50 uls of 1 M H_2SO_4 to each well. Read absorbance of yellow colored end-product by a microwell plate reader at 450nm/492nm.

The protocol described above may be utilized on a commercial basis by assembling in a kit the materials, polypeptides of the instant invention and reagents required to detect antibodies raised against HCV in biological samples, along with appropriate instruction.

Example II

Assay of Coded HCV Panel I

A coded panel containing 44 serum samples that have been tested by the kit from Ortho Diagnostics Co., Raritan, NJ, and the RIBA I (Recombinant ImmunoBlot Assay) and neutralization assays were received from The Center for Blood Research, 1631 Stockton Boulevard, Suite 250, Sacramento, California 95816-7089. At the time the coded HCV panel was received, the results of these above-mentioned tests on the 44 serum samples were unavailable. The 44 serum samples were tested with an ELISA utilizing Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides, as described in Example I. The Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides were chemically synthesized as described hereinbefore and included a cysteine amino acid residue at the amino termini and a tyrosine amino acid residue at the carboxyl termini. The results, expressed as absorbance at 450 nm, are recited in Table 8 (the cutoff value was set at 1.0, derived by addition of 0.4 to the mean of 5 negative controls), and are compared with those obtained at the Center for Blood Research, Sacramento in Tables 9 and 10. Of the two polypeptides, Cys-FGB1-Tyr appears to correlate better with those of the Ortho and RIBA I tests. The results are in agreement with those obtained at the Center for

Blood Research, Sacramento, except for sample =40. While sample =40 initially tested as reactive, when retested, however, it was non-reactive (NR). Sample #40 was a cross with samples =12 and =20, meaning that it was the same serum sample placed in three different tubes in the coded panel.

There are advantages for using the ELISA of the instant invention such as described in Example I versus the assays that were used by the Center for Blood Research, Sacramento, as mentioned herein. As observed in Table 9, forty-one percent (41%), i.e., eighteen out of forty-four samples (4, 5, 9, 11, 12, 18, 19, 20, 24, 33, 37, 38, 39, 40, 41, 42, 43 and 44), are false positives due to inconsistences between the Ortho and the RIBA I test. The results of the ELISA of this Example II correspond well with the RIBA I test, as evidenced by Tables 9 and 10.

Of significance are samples =6 and =13. These two samples were derived from a patient with clinically diagnosed NANBH, but tested negative with the Ortho test and RIBA I assay for HCV. While both samples were positive with the FGB1 ELISA test of the instant invention as shown in Table 9 (Cys-FGB1-Tyr), one sample was positive with the FGB2 ELISA test of the present invention as shown in Table 10 (Cys-FGB2-Tyr). This suggests that the ELISA tests and in particular the FGB1 ELISA test of the present

invention appear to be effective in the diagnosis of HCV due to acute or chronic phase infection. In Tables 9 and 10, R means reactive, NR means non-reactive, NT means not tested, I means indeterminant, Y means yes or positive, and N means no or negative.

The advantages of using the FGB1 and FGB2 polypeptides of the instant invention in an ELISA test are numerous. First, it is believed that the FGB1 and FGB2 polypeptides and espeically the FGB1 polypeptide have an increase in specificity and sensitivity, as compared to Ortho's assay which is concerned with the recombinant C100 peptide (antigen). It is also believed that assays involving the FGB1 and FGB2 are less expensive, and are believed to be capable of diagnosing HCV in the acute phase. Further, the polypeptide FGB1 is water soluble, very stable and binds readily to microwells of ELISA plates in an unconjugated form. Still further, since the FGB1 and FGB2 polypeptides can be prepared by chemical synthesis, they are easier to produce on a large scale without the variability found with recombinant proteins. Still further, the tests involving the FGB1 and FGB2 polypeptides are quantitative, allowing one to monitor the progress of the disease.

TABLE 8

No.	Cys-FGB1-Tyr	Cys-FGB2-Tyr
1	0.531	1.653
2	0.762	0.606
3	2.632	2.530
4	0.614	0.782
5	0.514	0.757
6	1.483	0.892
7	2.030	0.988
8	0.312	2.417
9	0.594	0.723
10	2.715	2.619
11	0.685	0.607
12	0.642	0.836
13	1.955	1.868
14	0.606	2.402
15	2.357	0.906
16	0.655	1.249
17	1.993	1.749
18	0.628	0.634
19	0.564	0.819
20	0.557	0.692
21	2.406	2.511
22	0.511	0.499
23	0.781	1.092
24	0.430	0.459
25	0.594	1.699
26	0.701	0.876
27	0.563	0.468
28	0.563	0.652
29	0.651	0.588
30	0.408	1.212
31	2.362	2.401
32	0.960	0.515
33	0.601	0.663
34	2.324	1.910
35	2.570	2.541
36	2.587	2.559
37	0.327	0.602
38	0.771	0.840
39	0.659	0.794
40	2.606	0.967
41	0.985	0.929
42	0.652	0.580
43	0.792	0.768
44	0.690	1.090

Cutoff Value = 1.0

TABLE 9

NO. Cys-FGB1-Tyr	CROSS1	CROSS2	ORTHO	RIBA	NEUT.	
1	8	25	NR	NT	NT	NR
2	28		NR	NT	NT	NR
3	10	31	R	R	Y	R
4	11	43	R	I	Y	NR
5	38	39	R	N	Y	NR
6	13		NR	N	NT	R
7			R	NT	NT	R
8	1	25	NR	NT	NT	NR
9	41	44	R	N	N	NR
10	3	31	R	R	Y	R
11	4	43	R	I	Y	NR
12	20	40	R	N	N	NR
13	6		NR	N	NT	R
14	29		NR	NT	NT	NR
15			R	NT	NT	R
16			NT	NT	NT	NR
17	34		R	R	Y	R
18	42	43	R	I	N	NR
19	24	37	R	N	Y	NR
20	12	40	R	N	N	NR
21	35	36	R	R	Y	R
22	27	32	NR	NT	NT	NR
23	30		NR	NT	NT	NR
24	19	37	R	N	Y	NR
25	1	8	NR	NT	NT	NR
26	16		NR	NT	NT	NR
27	22	32	NR	NT	NT	NR
28	2		NR	NT	NT	NR
29	14		NR	NT	NT	NR
30	23		NR	NT	NT	NR
31	3	10	R	R	Y	R
32	22	27	NR	NT	NT	NR
33	18	42	R	I	N	NR
34	17		R	R	Y	R
35	21	36	R	R	Y	R
36	21	35	R	R	Y	R
37	19	24	R	N	Y	NR
38	5	39	R	N	Y	NR
39	5	38	R	N	Y	NR
40	12	20	R	N	N	R
41	9	44	R	N	N	NR
42	18	33	R	I	N	NR
43	4	11	R	I	Y	NR
44	9	41	R	N	N	NR

TABLE 10

NO.	CROSS1 Cys-FGB2-Tyr	CROSS2	ORTHO	RIBA	NEUT.	
1	8	25	NR	NT	NT	R
2	28		NR	NT	NT	NR
3	10	31	R	R	Y	R
4	11	43	R	I	Y	NR
5	38	39	R	N	Y	NR
6	13		NR	N	NT	NR
7			R	NT	NT	NR
8	1	25	NR	NT	NT	R
9	41	44	R	N	N	NR
10	3	31	R	R	Y	R
11	4	43	R	I	Y	NR
12	20	40	R	N	N	NR
13	6		NR	N	NT	R
14	29		NR	NT	NT	R
15			R	NT	NT	NR
16			NT	NT	NT	R
17	34		R	R	Y	R
18	42	43	R	I	N	NR
19	24	37	R	N	Y	NR
20	12	40	R	N	N	NR
21	35	36	R	R	Y	R
22	27	32	NR	NT	NT	NR
23	30		NR	NT	NT	R
24	19	37	R	N	Y	NR
25	1	8	NR	NT	NT	R
26	16		NR	NT	NT	NR
27	22	32	NR	NT	NT	NR
28	2		NR	NT	NT	NR
29	14		NR	NT	NT	NR
30	23		NR	NT	NT	R
31	3	10	R	R	Y	R
32	22	27	NR	NT	NT	NR
33	18	42	R	I	N	NR
34	17		R	R	Y	R
35	21	36	R	R	Y	R
36	21	35	R	R	Y	R
37	19	24	R	N	Y	NR
38	5	39	R	N	Y	NR
39	5	38	R	N	Y	NR
40	12	20	R	N	N	NR
41	9	44	R	N	N	NR
42	18	33	R	I	N	NR
43	4	11	R	I	Y	NR
44	9	41	R	N	N	R

Example III

Generation of Anti-Cys-FGB1-Tyr and Anti-Cys-FGB2-Tyr Polypeptide Antibodies (Polyclonal) in Rabbits

The Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides were coupled to KLH, a carrier protein, with m-Maleimidobenzoyl-N- hydroxysuccinimide ester (MBS) and administered to separate rabbits as described below. Before coupling, however, a cysteine amino acid residue was added to the amino end and a tyrosine amino acid residue was added to the carboxyl end of each polypeptide according to standard techniques as for example described hereinbefore. This procedure is an established one to produce polyclonal antibodies to peptides in rabbits, and can be modified for use in other laboratory animals. Coupling of the Cys-FGB1-Tyr and Cys-FGB2-Tyr polypeptides to carrier proteins with m-Maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) was done in accordane with Kitagawa and Aikawa: J. Biochem., 79:233-236 (1976), Lia et al.: Biochemistry, 18:690-697 (1979), and Green et al.: Cell, 28:477-487 (1982).

A.) Materials and Reagents

1. 10mM phosphate buffer pH 7.0 (1.6 gm KH_2PO_4 to 1 L, adjust pH with NaOH)
2. 50 mM phosphate buffer pH 6.0 (6.8 gm KH_2PO_4 to 1 L, adjust pH with NaOH)

3. 15 mg/ml solution of MBS in dimethyl fomamide (DMF)

4. PBS 0.15 M NaCl in 10 mM phosphate buffer pH 7.3

5. MBS from Pierce, P.O. Box 117, Rockford, IL 61105.

6. DMF from Fisher, 711 Forbes Ave., Pittsburgh, PA 15219.

7. Keyhole limpet Protein (KLH) from Pierce, P.O. Box 117, Rockford, IL 61105.

8. Ovalbumin from Pierce, P.O. Box 117, Rockford, IL 61105.

9. Sephadex PD-10 from Pharmacia, 800 Centennial Ave., Piccataway, NJ 08854.

10. PBS from Sigma, P.O. Box 14508, St. Louis, MO 63178.

B.) Coupling to KLH (may be substituted with any other effective carrier)

Dissolve about 20 mg KLH in about 1.25 ml of about 10 mM phosphate buffer about pH 7.0 and stir overnight at room temperature. Spin down, remove supernatent. Read absorbance at 280 nm. Estimate the amount of KLH per ml using the ratio of 1 A_{280} which corresponds to about 2.26 mg KLH per ml. Dilute about 5 mg soluble KLH to about 500 ul in the 10 mM phophate buffer pH 7.0. Dissolve about 1-2 mg MBS at about 15 mg/ml in DMF. Add about 63 ul MBS solution dropwise with stirring to KLH solution. Stir the solution for about 30 minutes at room temperature. Load the solution on G-25 column equilibrated with about 50 mM phosphate buffer about pH 6.0 using the sephadex PD-10 column. Dissolve about 5.0 mg pure polypeptide in

about 1.0 ml PBS. Add the MBS-KLH pool off the G-25 column and adjust pH to about 7.0-7.5 with NaOH. Dialyze against PBS to remove uncoupled polypeptides. Stir at room temperature for about 3 hours. Divide the 1.5 ml dialized solution into 4 aliquots of about 0.5 ml and 1 each of about 0.33 ml.

C.) Injection of Rabbits

New Zealand rabbits obtained for the purpose of immunization are quarantined for a period of two weeks and are then injected and bled as follows.

DAY 01 Pre-bled and inject with a homogeneous emulsion of about 0.5 ml of coupled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide mixed with about 0.5 mg of Freunds Adjuvant and formed.

DAY 14 Inject with about 0.33 ml of the coupled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide mixed with about 0.3 mg of Freunds Adjuvant.

DAY 28 Boost again with about 0.33 ml of the coupled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide mixed with about 9.3 mg of Freunds Adjuvant and test bled.

DAY 60 Boost again with about 0.33 ml of the coupled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide mixed with about 0.3 mg of Freunds Adjuvant and test bled.

D. Testing of the Anti-Cys-FGB1-Tyr or Anti-Cys-FGB2-Tyr Polypeptide Antibodies in Rabbits

The procedure that may be used is essentially the same as that for testing of patient serum described in Example II, and the results are reported in Tables 11 and 12. Tables 11 and 12 show rabbit serum dilutions and corresponding absorbance readings in an ELISA using the respective Cys-FGB1-Tyr (Table 11) and Cys-FGB2-Tyr (Table 12) polypeptides.

TABLE 11

SERUM DILUTION	PRE-BLEED Cys-FGB1-Tyr A_{450}	3 MONTH BLEED Cys-FGB1-Tyr A_{450}		AVERAGE OF 3MO.DUP.-PREBLEED
1/10,000	0.226	2.703	2.667	2.459
1/25,000	0.142	2.659	2.667	2.521
1/50,000	0.169	2.659	2.639	2.480
1/75,000	0.154	2.620	2.639	2.475
1/100,000	0.134	2.620	2.588	2.470
1/250,000	0.149	2.218	2.153	2.036
1/375,000	0.167	1.775	2.051	1.746
1/500,000	0.193	1.384	1.685	1.341
1/750,000	0.185	1.087	1.277	0.997
1/1,000,000	0.252	0.806	0.753	0.527

TABLE 12

SERUM DILUTION	PRE-BLEED Cys-FGB1-Tyr A_{450}	3 MONTH BLEED Cys-FGB1-Tyr A_{450}		AVERAGE OF 3MO.DUP.-PREBLEED
1/10,000	0.112	2.320	2.254	2.175
1/25,000	0.088	2.330	2.342	2.248
1/50,000	0.102	2.362	2.131	2.145
1/75,000	0.083	2.158	2.166	2.079
1/100,000	0.074	2.077	2.332	2.130
1/250,000	0.067	1.782	1.771	1.710
1/375,000	0.068	1.477	1.513	1.427
1/500,000	0.085	1.321	1.548	1.350
1/750,000	0.071	1.173	1.135	1.083
1/1,000,000	0.084	0.806	0.805	0.721

Example IV

Monoclonal Antibodies to HCV

The use of spleen from rabits injected with immunogenic HCV polypeptides and in particular either the FGB1 or FGB2 polypeptides of the instant invention for raising monoclonal antibodies (MAbs) can be accomplished by, for example, the following procedures.

1. Hybridoma Technology

Monoclonal antibodies (MAbs) can be generated in accordance with standard technology, as recited for instance in Harlow and Lowe: Antibodies, A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1988). More particularly, the MAbs can be prepared by the fusion of myeloma cells with B-lymphocytes obtained from spleens of, for example, mice or other animals injected with for instance either the FGB1 or the FGB2 polypeptide conjugated with a carrier. In the presence of hypoxanthine, aminopterin and thymidine (HAT) medium, a few immortal hybridoma cells secreting specific immunoglobins can be selected in accordance with the standard techniques. These hybridomas can be further subdivided into clones by serial dilution of the cultured fusion products in 96 well microtiter plates in the presence of HAT media. The detail procedure

can be carried out as described hereinbefore. Monoclonal antibodies on conjugate groups to toxins (such as pseudomonas endotoxin), drugs, isotopes, dyes or the like could be used as diagnostics and/or in therapy.

2. ImmunoZAP™ Technology

Monoclonal antibodies can also be generated by a four step procedure. The first and second steps involve the construction and separation of heavy and light chain cDNA libraries from poly A mRNA isolated from spleen tissue, peripheral blood, or lymphnode derived from animals inoculated with, for example, the FGB1 or FGB2 polypeptides. The third and fourth steps involve randomly combining the heavy and light chain libraries and screening for antigen binding. This procedure is outlined in detail in the following publications: Antibody Expression Library Technology, distributed by Stratacyte, 11099 N. Torrey Pines Road, Suite 400, La Jolla, CA 92037; Marx, J.: Science, 246:1250-1251 (Dec. 8, 1989); and Huse, W.D. et al.: Science, 246:1275-1281 (Dec. 8, 1989).

Example V

1. Construction of Vaccinia Recombinant Viruses Containing Nucleotide Sequences which Encode the Immunogenic HCV Polypeptides and in Particular the FGB1 and/or FGB2 Polypeptides

(a) Construciton of Recombinant Plasmids.

Plasmids are constructed using existing vectors such as pSC11, Chakrabarti et al.: Molecular and Cell Biology, 5:3403-3409 (1985), pUV1, Falkner et al.: Nucleic Acids Res., 15:7192 (1987), pTM3, Moss et al.: Nature, (348):91-92 (1990), depending on the level of expression desired and on whether a stable recombinant virus is required. All three vectors are pUC based with the vaccinia virus thymidine kinase gene flanking inserted sequences. pSC11 may have, for example, the FGB1 and/or FGB2 polypeptides or other polypeptides of the instant invention expressed from an early/late promoter. pUV1 may have for instance the FGB1 and/or FGB2 polypeptides under the control of a strong late promoter. pTM3 has a bacteriophage T7 promoter and an EMC leader and is believed to be the best available vector for transient expression either in a cell line expressing T7 RNA polymerase or by coinfection of the cells with two viruses, on carrying the T7 RNA polymerase and the other carrying, for example, the FGB1 and/or FGB2 polypeptide of interest under the control of the T7 promoter. In addition, vectors are constructed from synthetic promoters whose

sequence is derived from mutagenesis studies of the promoter. The DNA encoding, for example, the FGB1 and/or FGB2 polypeptides are mutagenized at the nucleotides corresponding to the NH_2-terminus and the COOH-terminus of the open readin frame (ORF) to have proper restriction sites for insertion into any particular vector.

(b) Generation of Vaccinia Virus Recombinants.

Vaccinia virus recombinants are generated as described previously in Kotwal, G. J. et al: Virology, 171:579-587 (1989). CV-1 cells in monolayers (25 cm^2 flask) are infected with about 1 ml of vaccinia virus at about 0.05 pfu. The virus inoculum is left on cells for about 2 h at about 37°C with rocking. Calcium phosphate precipitated DNA is prepared by gently mixing about 5-15 ug of plasmid DNA in about 1 ml of hepes buffered saline, pH about 7.05, (NaCl 0.14 M, KCl 5 mM, $Na_2HPO_4*2H_2O$ 1 mM, Dextrose 0.1%, Hepes 20 mM). About 50 ul of about 2.5 mM $CaCl_2$ is added and after gentle mixing is left at room temperature for about 30 min. The virus inoculum is aspirated off and about 1 ml of the DNA precipitate is placed over the cells at room temperature for about 30 min. About 9 ml of prewarmed Eagles MEM containing about 8% FBS is then added to the DNA precipitate and the incubation is carried out for about 3.5 h at about

37°C. The medium is aspirated off and replaced with about 10 mls of fresh Eagles MEM containing about 8% FBS and left at about 37°C for 2 days. After about 48 h the cells are harvested by scraping from bottles, and resuspended in about 0.5 ml Eagles MEM and frozen at about -20°C.

(c) Selection of Vaccinia Virus Recominants.

Depending on the vector used, the recombinants are picked off either TK-cells in the presence of 6-bromouridine, Chakrabarti et al: Mol. Cell. Biol., 5:3403-3409 (1985), or in the presence of mycophenolic acid if the gpt gene is inserted in tandem with the foreign gene. Blue color selection is used with lac Z containing vectors, Falkner and Moss: J. Virol., 62:1849-1854 (1988).

2. Large Scale Production of Recombinant Vaccinia Viruses.

(a) Growth and Purification of Vaccinia Virus Recombinants.

Recombinant vaccinia viruses are purified according to Joklik: Virology, 18:9-18, (1962). HeLa cells are grown in suspension in MEM spinner medium containing about 5% horse serum in large Bellco 36L spinner flasks with an overhead drive and a teflon paddle impeller assembly at a density of about $5x10^5$ cells/ml. Up to about 100 1 of HeLa cells are then

infected with trypsinized crude virus made under selective conditions at a multiplicity of infection of about 10 and at a cell density of about $2x10^7$ cells/ml for about ½ hour. After about ½ hour, the cell density is brought back to about $5x10^5$ cells/ml and is incubated at about 37°C for about 48 hours. At the end of about 48 hours, the cells are harvested, washed in PBS and resuspended in about 1/10 volumes of about 10mM Tris HCl (pH 9.0) and homogenized in a douce homogenizer placed in ice using about 25 strokes, five at a time. The homogenate is then centrifuged at about 2500 rpm for about five minutes. The supernatant is then vortexed and sonicated for about 60 seconds and placed on an equal volume of about 36% sucrose and centrifued at about 13,500 rpm in a SW27 rotor for about 80 minutes. The pellet is resuspended in about 10mM Tris HCl (pH 9.0), sonicated and placed over a sterile sucrose step gradient (ranging from 25% - 40%) left over-night at about 4°C. The gradient is then spun in SW 27 rotor at about 12,000 rpm for about 45 minutes. The virus band is then aspirated and repelleted in about 25% sucrose by spinning in an SW 27 rotor at about 13,500 rpm for about 60 minutes. The virus pellet is then resuspended in about 1mM Tris HCl (pH 9.0) and distributed into screwtop microfuge tubes in about 150ul aliquots. A titer of about 1 x 10^{11}/ml is the yield expected for each titer of cells.

(b) Quantitation of Virus.

The following conversion table is used for estimating the amount of virus particles:

$1\ OD_{260}$ = 64 ug virus particles
$1\ OD_{260}$ = 2.5 ug DNA

(c) Titration of Virus.

The virus is titered on BSC-1 cells under selective and nonselective conditions. The titer is read at about 48 hours after different dilutions of the virus are placed on confluent BSC-1 cells on a 6 well plate.

(d) Southern Blot Analysis.

DNA is estimated from the absorbance of the virus preparation and is isolated by phenol:chloroform extraction of the proteinase K treated and detergent lysed suspension. It is then digested by Hind III and SAl I and transferred to nitrocellulose and probed with the nick translated FGB1 and/or FGB2 polynucleotide sequence that is expected to be inserted in the genome. Both its presence and location are determined. Essentially the protocol of Sambrook, J. et al.: Molecular Cloning. A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) is followed.

(e) Western Blot Analysis (WBA) and/or Immunofluorescence (IF).

CV-1 cells are infected at an m.o.i. of 30 (for WBA) and an m.o.i. of 10 (for IF) and the lysed cells are separated by 12.5% PAGE, transferred to nitrocellulose, Sambrook, J. et al.: Molecular Cloning. A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989), and probed with patient's serum or specific antibody if available. In case of immunofluorescence, the cells are grown on 8 well slides and 24 hour post infection treated with the antibody and then with FITC conjugated antibody, Kotwal, G.J. et al.: Modern Approaches to New Vaccines Including Prevention of AIDS, pp. 389-392, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989). The fluorescence indicates the intracellular localization of, for example, the FGB1 and/or FGB2 polypeptides.

Example VI

Radioimmuno Assay (RIA)

A.) Detection of Antibodies against HCV in Sera

Purified IgG antibody is obtained from the hyperimmune serum (polyclonal antibody) of Example III. More particularly, the hyperimmune sera, i.e., either anti-Cys-FGB1-Tyr or anti-FGB2-Tyr, obtained from the hyperimmunized rabbits are obtained and subjected to ammonium sulfate precipitation, and the precipitate is obtained as an IgG fraction. The obtained IgG fraction is subjected to gel chromatography using Sephacryl S200, distributed by Pharmacia Fine Chemicals AB, Sweden, to obtain a purified anti-Cys-FGB1-Tyr IgG or anti-Cys-FGB2-Tyr IgG (anti-FGB IgG). The purified anti-FGB IgG is labeled with, for example, ^{125}I by the chloramine T method, as described in Greenwood et al.: Biochemical J., 80:114 (1963). Illustratively stated, about 4 ml of the purified anti-FGB IgG (5 mg/ml) is cooled in ice water, and about 1 mCi/10 ul of ^{125}I is added to the purified anti-FGB IgG while gently stirring. Then, about 1 ml of about 200 ug/ml aqueous chloramine T is dropwise added to the mixture and stirred for about 5 minutes to advance a reaction. About 1 ml of about 200 ug/ml aqueous sodium methabisulfite is added to the resultant mixture to terminate the reaction. The resultant mixture is dialyzed to remove the

unreacted ^{125}I, to thereby obtain ^{125}I-labeled anti-FGB IgG.

Using the above obtained ^{125}I-labeled anti-FGB IgG and the appropriate Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide, an antibody against HCV in serum samples obtained from patients having HCV hepatitis can be detected by radioimmuno assay (RIA). This can be accomplished by, for example, binding about 0.1 ml of appropriate Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide (1 ug/ml) to each well of the reaction plate for the RIA. Thereafter, the above solution is replaced with about 200 ul of PBS:Tween (Example I), and further introducing about 0.1 ml of a serum sample to all but three control wells of the reaction plate. In one of the three serum-free control wells of the reaction plate, about 0.1 ml of a control serum positive for HCV is added. In a second serum-free control well, about 0.1 ml of a control serum negative for HCV is added. After about 4 hours of incubation at room temperature, the serum samples are removed and the reaction plate is washed several times with PBS:Tween. To all wells of the reaction plate, about 0.1 ml of the appropriate ^{125}I-labeled anti-FGB IgG is added and allowed to stand overnight at room temperature to advance a reaction. The resultant wells are sufficiently washed with PBS: Tween. The radioactivity (CPM) of each well is

measured using, for example, an auto-gamma counter. From the radioactivity, an inhibition (%) of the reaction between the appropriate ^{125}I-labeled anti-FGB IgG and the Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide bound in the wells is calculated using standard techniques.

When the serum samples contain antibody against HCV, the reaction between the appropriate polypeptide bound to the wells and the ^{125}I-labeled anti-FGB IgG is inhibited by the reaction between the polypeptide bound in the wells and the anti-HCV antibody present in the serum. Therefore, the radioactivity of the wells ascribed to the labeled antibody bonded to the appropriate polypeptide bound in the wells will be relatively low in those serum samples derived from patients having antibodies against HCV. This can be confirmed by comparing the radioactivity between the serum wells and the three control wells.

B.) Detection of HCV Antigen in Sera

^{125}I-labeled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide and anti-FGB-IgG bound in the wells of the reaction plate are prepared in substantially the same manner as mentioned above. The appropriate Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide is labeled with, for example, ^{125}I by the chloramine T method

outlined in Greenwood, et al.: Biochemical J., 80:114 (1963). The anti-FGB-IgG are bound to the wells by simply adding the appropriate anti-FGB-IgG to the wells of the reaction plate.

Using the above-obtained ^{125}I-labeled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide, and the appropriate anti-FGB-IgG antibody, HCV antigen present in serum samples obtained from patients infected with HCV hepatitis can be detected in substantially the same manner as mentioned above.

This is accomplished by, for example, introducing about 0.1 ml of the appropriate anti-FGB-IgG in each well of the reaction plate for RIA. Thereafter replacing the above solution with about 200 ul PBS:Tween (Example I), and further introducing about 0.1 ml of a serum to all but three control wells of the reaction plate. In one of the three serum-free control wells of the reaction plate, about 0.1 ml of a control serum positive for HCV is added. In a second serum-free control well, about 0.1 ml of a control serum negative for HCV is added. After about 4 hrs. of incubating at room temperature, the serum samples are removed from the wells and the plate is washed several times in PBS:Tween. To all wells of the reaction plate, about 0.1 ml of the appropriate ^{125}I-labeled-Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide (1 ug/ml) is added and allowed to stand

overnight at room temperature to advance a reaction. The resultant wells are sufficiently washed with PBS:Tween. The radioactivity (CPM) of each well is measured using, for example, an auto-gamma counter. From the radioactivity, an inhibition (%) of the reaction between the appropriate ^{125}I-labeled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide and the appropriate anti-FGB-IgG bound to the wells is calculated using standard techniques.

When the serum samples contain HCV antigen, the reaction between the ^{125}I-labeled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide and the appropriate anti-FGB-IgG bound to the wells is inhibited by the reaction between the HCV antigen and the anti-FGB-IgG bound in the wells. Therefore, the radioactivity of the wells ascribed to the ^{125}I-labeled Cys-FGB1-Tyr or Cys-FGB2-Tyr polypeptide bonded to the anti-FGB-IgG bound to the wells will be relatively low in those samples derived from patients having HCV antigen. This can be confirmed by comparing the radioactivity between the serum wells and the three control wells.

Example VII

FGB1 Clone

Two polynucleotide DNA sequences designated as GB1 and GB2, respectively, each 117 nucleotides in length are synthesized on a Gene Assembler Plus (Pharmacia LKB, Sweden) in accordance with the Pharmacia Manual and reagents supplied therewith. The polynucleotide sequences for GB1 and GB2 are as follows:

SEQ ID NOS: 31-31

GB1 5'-GAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGATT
CCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCA
CAGGACGTCTAGTTCCCGGGTGGCGGT-3'; and

GB2 5'-CTCACGGGGCCCTCCAGAGCATCTGGCACGTGGTACTCGTGCTAA
GGGTTTGGAGTTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGT
GTCCTGCAGATCAAGGGCCCACCGCCA-3'.

See also Table 13(GB1) and Table 14(GB2). Polynucleotide GB2 is the complementary sequence of GB1. The GB1 and GB2 polynucleotides are annealed according to the methods of Sambrook, J., et al.: Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., (1989), with slight modifications. Approximately 5-10 ng/ml of final concentration of GB1 and GB2 are denatured in about 5 mls of annealing buffer (0.1 M NaCl, 10 mM Tris-Cl (pH 7.8), 1.0 mM EDTA) at about

95° C. for about 5 minutes and allowed to cool at room temperature. Two volumes (about 10 mls) of 100% ethanol are added and the annealed polynucleotides (GB1-GB2) are allowed to precipitate overnight at about -20° C. The annealed polynucleotides (GB1-GB2) are centrifuged at about 14,000 x g, dried and resuspended in about 100 ul of sterile water. About 1.0 ul of the annealed polynucleotides is treated with S1 Nuclease (BRL, Gaithersburg, MD under catalog nos. 80015A or 80015B) and electrophoresed on a 1.5% agarose gel next to a 1.0 ul control sample that is not subjected to treatment with S1 Nuclease. S1 Nuclease is a single stranded specific endonuclease which hydrolyzes single stranded RNA or DNA into 5'-mononucleotides. The result of the electrophoresis indicates that approximately 90-95% of the GB1, GB2 is annealed yielding the desired double stranded (ds) GB1-GB2 fragment. The sequence of the annealed ds GB1-GB2 fragment contains two Sma I restriction enzyme sites, CCCGGG, which are 98 nucleotides apart as well as an initiation codon ATG and a stop codon TAG, both of which are positioned between the two Sma I restriction enzyme sites. In addition, the annealed ds GB1-GB2 fragment contains the polynucleotide sequence which encodes the FGB1 polypeptide, and flanking nucleotide sequences which

lie upstream and downstream from the respective 5′ end and 3′ end Sma I restriction sites.

Annealed ds GB1-GB2 Fragment

SEQ ID NOS: 33-34

```
                    Sma I
                      ↓
GB1  5'-(GAGTGC)CCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACG
GB2  3'-(CTCACG)GGGCCCTCCAGAGCATCTGGCACGTGGTACTCGTGC

GB1  ATTCCCAAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCC
GB2  TAAGGGTTTGGAGTTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGG

                          Sma I
                            ↓
GB1  ACAGGACGTCTAGTTCCCGGG(TGGCGGT)-3'
GB2  TGTCCTGCAGATCAAGGGCCC(ACCGCCA)-5'
```

The two underscored polynucleotide sequences designated as CCCGGG in the above annealed ds GB1-GB2 represent restriction sites for the enzyme Sma I. The two restriction sites CCCGGG are digested by the Sma I enzyme (BRL, Gaithersburg, MD, catalog no. 52285A) between the CCC and GGG nucleotides as indicated in the above-recited annealed ds GB1-GB2 fragment. The 6 complementary nucelotide sequence in parentheticals (GAGTGC) (CTCACG) represents the flanking complementary nucleotide sequence which lies upstream from the 5' end of the Sma I restriction site whereas the 7 complementary nucleotide sequence in parentheticals (TGGCGGT) (ACCGCCA) represents the flanking complementary nucleotide sequence which lies downstream from the 3' end Sma I restriction site. Moreover, the polynucleotide sequences ATG and TAG represent initiation and stop codons, respectively. The annealed ds GB1-GB2 fragment between the

initiation codon ATG and termination codon TAG encodes a polypeptide which includes the amino acid sequence for the FGB1 polypeptide. It should be understood that the polynucleotide sequence in the above annealed ds GB1-GB2 fragment which lies between the 5' end restriction site CCCGGG and the initiation codon ATG is optional, and therefore can be deleted, modified, shortened or lengthened so long as such modification(s) does not cause the annealed ds GB1-GB2 fragment encoding FGB1 to be out of phase. In addition, other restriction sites and other flanking sequences may be employed so long as they permit the annealed ds GB1-GB2 fragment to be ligated into a replicating expression vector and do not cause the annealed ds GB1-GB2 fragment encoding the polypeptide which contains the amino acid sequence of FGB1 to be out of phase.

The above annealed ds GB1-GB2 is digested with Sma I restriction enzyme and purified as a 98 nucleotide ds fragment from a low melt 1.5% agarose gel containing about 1 ug of Ethidium Bromide (International Biotechnologies, Inc., New Haven, CT, catalog no. 40060) per ml of agarose (FMC Bioproducts, Rockland, ME, catalog no. 50004) in TAE buffer (0.04 M Tris-acetate 0.001 M EDTA). After the electrophoresis is stopped, long wavelength ultraviolet light is shined over the gel and the band containing the 98 bp

Sma I-Sma I fragment is cut with a razor blade into small pieces and introduced into a 17 x 100 mm polypropylene round bottom tube (Becton Dickinson Labware, Lincoln Park, NJ, catalog no. 2059). The tube with the small fragments is weighed to determine the net weight of the fragments (came up to 2.4 g). About 12 ml of the TE buffer (10mM Tris-HCl, pH 7.5, 0.1 mM EDTA) is added to this tube and placed in a water bath at about 65°C for about 15 minutes with occasional shaking. After 15 minutes, the tube is transferred to the bench and allowed to reach room temperature in about 0.75 hr. At this time, about 0.4 ml of sodium acetate (Mallinckrodt, Paris, KY) is added. Half of the contents of this tube is transferred into another identical tube and about 7 ml of phenol (BRL Life Technologies, Inc., Gaithersburg, MD catalog no. 5509UA) is added to each tube and the contents shaken vigorously for about 3 minutes at which time they are centrifuged in a Beckman GPR Centrifuge (Beckman Instruments, Inc., Spinco Division, Palto Alto, CA catalog no. 349702) at about 3800 rpm for about 5 minutes. The aqueous layer is removed and transferred into a new tube and 7 ml of a 50/50 mixture of phenol and chloroform is added to each tube and again shaken vigorously and centrifuged as above. Again the aqueous layer is removed and transferred into a new tube and about 7 ml of

chloroform (Mallinckrodt, Paris, KY) is added to each tube and again shaken vigorously and centrifuged as above. The aqueous layers from both tubes are pooled together (13 ml total, some loss occurs in each step of the way) and about 1 ml of about 0.3 M sodium acetate (Mallinckrodt, Paris, KY) is added and the total volume of about 14 ml is transferred to four tubes (about 3.5 ml/tube). To each of the four tubes, about 7 mls of 100% ethanol is added, shaken and allowed to sit at about -20°C overnight. The four tubes are then centrifuged at about 4°C for about 30 minutes in a beckman Model J2-21M Induction Drive Centrifuge (Beckman Instruments, Inc. Spinco Division, Palto Alto, CA catalog no. 341737). At the end of centrifugation, the liquid portion in each tube is discarded and the pellet is allowed to dry at room temperature. The pellets in the four tubes are combined in a total of about 40 ul of water, about 1.0 ul of which is subjected to electrophoresis on 1.5% agarose gel with standard markers in order to estimate the purity and yield of this particular batch of the 98 bp SmaI-Sma I fragment.

Sma I-Sma I
98 Base Pair Fragment

SEQ ID NOS: 35-36

GB1 5'-GGGAGGTCTCGTAGACCGTGCACCATGAGCACGATTCCCAAACCTC
GB2 3'-CCCTCCAGAGCATCTGGCACGTGGTACTCGTGCTAAGGGTTTGGAG

GB1 AAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAGGACGTCTA
GB2 TTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGTGTCCTGCAGAT

GB1 GTTCCC-5'
GB2 CAAGGG-3'

The Sma I-Sma I 98 base pair fragment, about 0.5 ug, is ligated into the Sma I site of the linearilized, dephosphorylated pGEM™-3Zf(-) vector, about 0.1 ug (Promega, Madison, WI, under catalog no. P2241), in ligation buffer (about 66 mM TRIS-Cl, about pH 7.6, about 6.6 mM $MgCl_2$, about 10 mM DTP, and about 66 uM ATP) in the presence of about 1 unit of T4 DNA Ligase (BRL, Gaithersburg, MD, catalog no. 5224SB) for about 4 hours at room temperature. After incubation, about 1 ul of the ligation mixture is then introduced into a competent, high efficiency strain of E. coli bacterial cells designated as JM 109 (Promega, Madison, WI, under catalog No. L2001), which have been stored at -70°C. Before introducing the ligated Sma I-Sma I 98 base pair fragment into the competent E. coli JM 109 cells, the competent E. coli cells are thawed on ice for about 30 min. Following thawing, the ligated ds material is mixed with the thawed competent cells for about 30 min. on ice prior to heat shock treatment. To heat shock, the tube containing the mixture is

placed in a water bath having a temperature at about 42°C for about 45 seconds. After heat shocking, about 900 ul of LB (Lauria -Bertani Medium, i.e., Peptone 140 10 g/l, and Select Yeast Extract 5 g/l, Gibco-BRL, Gaithersburg, MD, catalog nos. M00392B and M00393B, respectively, and Sodium Chloride 10 g/l, Sigma, P.O. Box 14508, St. Louis, MO 63178, catalog no. S3014) is added to the heat shocked mixture and allowed to incubate at about 37°C for about 60 min. Thereafter, about 100 ul of the bacteria is plated out on agar ampicillin LB plates and incubated at about 37°C overnight to generate transformants containing the FGB1 recombinant plasmid, i.e., the FGB1 clones.

The agar ampicillin LB plates are made as follows and in accordance with Sambrook, J. et al.: Molecular Cloning. A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) with slight modification. Prior to autoclaving, about 15 g of Select Agar (Gibco-BRL, Gaithersburg, MD, catalog no. M00391B) is added to about 1.0 liter of LB. The liquid media in the flask is autoclaved for about 45 min. and then allowed to equilibrate in a water bath to about 58°C before adding the ampicillin thereto. Once equilibrated, ampicillin is added to the liquid media to a final concentration of about 100 ug/ml. The agar ampicillin LB plates are prepared by pouring the liquid media

from the flask into the plates. Approximately 30-35 ml of liquid media is poured into each 85-mm petri dish. If bubbles occur, the surface of the liquid media can be flamed with a bunsen burner before solidification of the agar. The agar ampicillin LB plates are stored at room temperature for approximately 1-2 days before use to minimize condensation and sweating. If excessive moisture is present, the bacterial colonies may streak. The ampicillin is prepared as follows. Ampicillin (obtained from International Biotechnologies, Inc., New Haven, CT, catalog no. 02040) is dissolved in water to make a stock solution having a concentration of about 50 mg/ml of the sodium salt of ampicillin. The ampicillin stock solution is sterilized by filtration and stored in selected 1.0 ml aliquots at about -20°C.

Following incubation, single colonies are randomly selected and allowed to grow in about 10 ml LB containing about 100 ug/ml ampicillin overnight at about 37°C with vigorous shaking (about 225 rpm). Mini-preparations are made of each cultivated colony in accordance with Sambrook, J. et al. Molecular Cloning, A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989) with slight modification. About 1.5 ml of the overnight culture is then poured into an Eppendorf

tube. The culture is centrifuged at about 14,000 rpm for about 1 min. in an Eppendorf centrifuge. The remainder of the overnight culture is stored at about 4°C. The centrifuged medium is removed by aspiration, leaving the bacterial pellet as dry as possible. The dried pellet is then resuspended in the same tube by vortexing in about 100 ul of an ice-cold solution of about 500 mM glucose, 10 mM EDTA and 25 mM Tris-Cl (about pH 8.0). The resuspended pellet is allowed to stand for about 5 min. at room temperature. To the Eppendorf tube, approximately 200 ul of a freshly prepared solution containing about 0.2 N NaOH and about 1% SDS is added. The Eppendorf tube is then closed and the contents thereof are mixed by inverting the Eppendorf tube quickly for about 2-3 min. Do not vortex the Eppendorf tube during this step. The Eppendorf tube is then stored on ice for approximately 5 min. At this point, about 150 ul of an ice-cold potassium acetate solution, about pH 4.8 (about 11.5 ml of glacial acetic acid and about 28.5 ml of water are added to about 60 ml of 5 M potassium acetate), is added to the Eppendorf tube. The potassium acetate solution is about 3 M potassium and 5 M acetate. The Eppendorf tube is then closed and mixed gently in an inverted position for about 10 seconds and then allowed to stand on ice for approximately 5 min. The Eppendorf tube is then centrifuged at about 14,000 rpm

for about 10 min. in an Eppendorf centrifuge at about 4°C. The supernatant is then transferred to a new Eppendorf tube. An equal volume of phenol is added to the Eppendorf tube and mixed by vortexing. The Eppendorf tube is then centrifuged at about 14,000 rpm for about 1 min. in an Eppendorf centrifuge, and the aqueous layer is transferred to another Eppendorf tube where an equal volume of chloroform is added and mixed by vortexing. The Eppendorf tube is centrifuged for about 1 min. in an Eppendorf centrifuge at about 14,000 rpm, and the aqueous layer is removed and transferred to a new Eppendorf tube. Two volumes of ethanol, about 1 ml, are added to the Eppendorf tube at room temperature and mixed by vortexing. The resultant solution is stored at -20°C for about 1 hr. Thereafter, the Eppendorf tube is centrifuged in an Eppendorf centrifuge at about 4°C for approximately 10 min. at about 14,000 rpm. The supernatant is then removed and the remaining contents in the Eppendorf tube are dried in a Speed Vac Concentrator (Model SVC 100H distribued by Savant Instruments, Inc., Farmandale, N.Y.). Approximately 20 ul of TE buffer (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA), containing about 20-40 ug/ml DNase-free pancreatic RNase (Boehringer Mannheim Biochemicals, Indianapolis, IN) is added to the dried pellet in the Eppendorf tube and is mixed briefly and allowed to incubate at about 37°C

for about 20 min. About 2 ul of the solution is then transferred to a new Eppendorf tube wherein about 2 ul of React 6 buffer (50 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, 50 mM KCl, 50 mM NaCl), 1 unit of Pvu II restrictive enzyme (BRL, Gaithersburg, MD, under catalog no. 54125A) and about 15 ul of water is added. The resultant solution is incubated for about 45 min. at about 37°C. to yield ds DNA fragments. The remainder of the untransferred preparation can be stored at about -20°C.

It should of course be appreciated by those of skill in this art that digestion of plasmids obtained by minipreparations yields ds DNA fragments that can be cleaved to completion with appropriate restriction enzymes. Nonetheless, since certain batches of some enzymes may be less effective with these minipreparations of the plasmids than with highly purified DNA, this may be overcome by increasing the amount of enzyme by two-three fold and/or by increasing the time of incubation by approximately 6-12 hrs.

The resultant ds DNA fragments after digestion with Pvu II can be analyzed by gel electrophoresis in 1% agarose gel (FMC Bioproducts, Rockland, ME, catalog no. 50004). The clones that contain the inserted HCV polynucleotide sequence will have one of its ds DNA fragments migrate with an

increase in mobility by about 98 nucleotides as compared with a Pvu II digest of pGEM^{TM}-3ZF(-) plasmids and standard DNA markers electrophoresed in the same 1% agarose gel. This confirms the insertion of the Sma I-Sma I 98 base pair fragment into the pGEM^{TM}-3ZF(-) plasmid and the existence of an FGB1 clone.

To further confirm the insertion of the Sma I-Sma I 98 base pair fragment into the pGEM^{TM}-3ZF(-) plasmid and the existence of the FGB1 clone, direct DNA sequencing of a single stranded clone designated as UR-23 using Reagent Kit for DNA Sequencing with Sequenase, United States Biochemical Corporation, Cleveland, OH, catalog no. 70721 (Sequenase T7 DNA Polymerase, U.S. Patent No. 4,795,699) is performed. To prepare the single stranded UR-23 clone, an overnight culture of a single colony containing pGEM^{TM}-3ZF(-) plasmid (Promega, Madison, WI, catalog no. P2261) with the Sma I-Sma I 98 nucleotides fragment insert is grown in about 50-100 ug/ml ampicillin (International Biotechnologies, Inc., New Haven, CT, catalog no. 02040). About 5 ml of LB with 50 ug/ml ampicillin is inoculated with about 100 ul of overnight culture. It is shaken for about 1-2 hours at 37°C at 225 rpm. The culture is infected with about 80 ul of about 5 x 10^{10} pfu/ml of helper phage R408 (Promega, Madison, WI,

catalog no. P2291), and the shaking is continued overnight at 225 rpm. Supernatant is collected by spining the cells at about 12,000 g for about 15 min. at about 4°C. The supernatant is removed, transferred to a new tube and recentrifuged at about 12,000 g for about 15 min. The phage is precipitated by adding about 0.25 ml volume of 20% PEG (Polyethylene glycol Sigma, St. Louis, MO, catalog no. P-5413) and about 2.5 M sodium chloride at room temperature for about 1 hr. and is then centrifuged at about 12,000 g for about 15 min. at about 4°C. The supernatant is discarded and the pellet is resuspended in about 400 ul of a buffer comprised of about 10 mM Tris-HCl pH 8.0 (International Biotechnologies, Inc., New Haven, CT, catalog no. 70142) and 0.1 mM EDTA (International Biotechnologies, Inc., New Haven, CT, catalog no. 70182). The resuspended pellet is first extracted with an equal volume of phenol (BRL Life Technologies, Inc., Gaithersburg, MD, catalog no. 5509UA) and then with an equal volume of chloroform (Mallinckrodt, Paris, KY). Several phenol/chloroform extractions are performed until the interface between the aqueous and organic phases is clear. DNA is precipitated by addition of about 1/10 volume of about 3 M Sodium Acetate (Mallinckrodt, Paris, KY) and about 2.5 ml of absolute ethanol (Aaper Alcohol and Chemical Company). Purity of the DNA of the single stranded UR-23 clone

is examined upon electrophoresis of about 2 ul on about 1.2% agarose gel (FMC Bioproducts, Rockland, ME, catalog no. 50004).

The results of the direct sequencing of the single stranded UR-23 clone reveal that the Sma I-Sma I 98 base pair fragment is inserted into the pGEM^{TM}-3ZF(-) plasmid, and that such plasmid is introduced into a competent, high efficiency E. coli bacterial cell of the JM 109 strain, but with slight modification. According to the direct sequencing results, two bases in the GB1 Sma I-Sma I 98 nucleotides fragment and their complements in the GB2 fragment are changed by the JM 109 bacterial cells. More particularly, the base pairs designated at the 20th and 66th positions downstream from the 5′ Sma I end of the transcribing GB1 strand are changed from a dGMP(G)-dCMP(C) to a TMP(T)-dAMP(A) and from a dCMP(C)-dGMP(G) to a dAMP(A)-TMP(T), respectively, as illustrated below:

Modified Sma I-Sma I 98 Base Pair Fragment and Translation Thereof

SEQ ID NOS: 37-39

```
     Sma I                (G)
                  10        20        30
GB1 5'-GGGAGGTCTCGTAGACCGTTCACCATGAGC
GB2 3'-CCCTCCAGAGCATCTGGCAAGTGGTACTCG
                          (C)   METSer

                  40        50        60
GB1     ACGATTCCCAAACCTCAAAGAAAAACCAAA
GB2     TGCTAAGGGTTTGGAGTTTCTTTTTGGTTT
        ThrIleProLysProGlnArgLysThrLys
                                          Sma I
            (C)  70        80        90
GB1     CGTAAAACCAACCGTCGCCCACAGGACGTCTAGTTCCC-3'
GB2     GCATTTTGGTTGGCAGCGGGTGTCCTGCAGATCAATTT-5'
            (G)
        ArgLysThrAsnArgArgProGlnAspValEND
          (Asn)
```

More particularly, in the modified Sma I-Sma I 98 base pair fragment, the base pairs designated in parentheticals (G)-(C) and (C)-(G) at the 20th and 66th positions represent the original base pairs which are changed in the GB1 sequence by the JM 109 bacterial cells. The two sequences ATG and TAG are the initiation and termination codons, respectively. The lysine amino acid residue in parentheticals (Asn) represents the original amino acid residue encoded by the GB1 strand. The amino acid residues underscored in the modified Sma I-Sma I 98 base pair fragment constitute those amino acid residues which correspond to the amino acid sequence for the FGB1 polypeptide, except for the substituted lysine amino acid residue (Lys).

The change at the 20th base pair from the 5′ Sma-I end in the transcribing GB1 strand of the modified Sma I-Sma-I 98 base pair fragment appears to be of little importance since it is positioned upstream from the ATG initiation codon. Nevertheless, the effect of the change at the 66th base pair from the 5′ Sma I end in the modified GB1 strand appears to be of greater significance since it changes a codon consisting of bases 64-66, which is positioned downstream from the ATG initiation codon, but upstream from the TAG termination codon. The modified codon in the FGB1 clone substitutes a lysine amino acid residue (Lys) for an asparagine amino acid residue (Asn).

It should be understood by those of skill in the art that the new dAMP(A)-TMP(T) base pair in the modified Sma I-Sma I 98 base pair fragment at position 66 may be converted back to the original dCMP(C)-dGMP(G) base pair by using the technique of site directed mutagenesis, provided that the JM-109 strain or other bacterial strains utilized will permit this change to remain stable in the newly developed FGB1 clone.

It, of course, should be understood that the present invention contemplates other FGB1 clones prepared by other recombinant techniques known in the art as well as other clones prepared with recombinant DNA fragments which encode amino acid sequences

comprising the polypeptides of the instant invention. Moreover, it should be understood that the instant invention contemplates FGB2 clones which may be prepared in a similar manner as mentioned hereinbefore.

TABLE 13

Gene Assembler Plus

Sequence: GB1
Synthesis: GB1
Scale: 0.2 micromole
Sequence Length: 117
Column: 1
Final Detritylation: Yes
Coupling Efficiency Threshold: 50%

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
117	T	0.38	0.70	477	34.96	--	--
116	G	0.35	0.62	475	43.97	--	--
115	G	0.36	0.67	547	51.28	116.6	100.0
114	C	0.36	0.77	568	64.76	--	100.0
113	G	0.35	0.67	538	52.60	101.3	100.0
112	G	0.35	0.70	550	56.54	107.5	100.0
111	T	0.40	0.82	614	69.29	--	100.0
110	G	0.38	0.71	588	55.48	99.1	100.0
109	G	0.37	0.69	589	56.27	101.4	100.0
108	G	0.36	0.67	537	52.88	94.0	100.0
107	C	0.41	0.81	607	67.51	100.6	100.0
106	C	0.39	0.79	580	66.18	98.0	100.0
105	C	0.42	0.79	559	62.69	94.7	100.0
104	T	0.40	0.83	593	68.67	99.9	100.0
103	T	0.43	0.84	605	72.75	105.9	100.0
102	G	0.41	0.71	513	53.94	100.3	100.0
101	A	0.39	0.77	572	62.04	--	100.0
100	T	0.43	0.84	605	71.56	99.5	100.0
99	C	0.41	0.82	606	70.32	101.9	100.0
98	T	0.43	0.83	615	70.16	99.0	100.0

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
97	G	0.40	0.71	529	53.72	99.9	100.0
96	C	0.45	0.83	544	65.17	97.5	100.0
95	A	0.42	0.80	566	63.56	100.4	100.0
94	G	0.40	0.73	510	53.94	100.1	100.0
93	G	0.42	0.73	507	52.32	97.0	100.0
92	A	0.45	0.75	522	60.86	98.6	100.0
91	C	0.43	0.82	543	62.23	99.1	100.0
90	A	0.45	0.78	527	59.68	99.0	100.0
89	C	0.44	0.84	563	63.72	101.2	100.0
88	C	0.46	0.84	549	64.18	100.7	100.0
87	C	0.43	0.84	500	62.71	97.7	100.0
86	G	0.45	0.74	485	50.21	99.4	100.0
85	C	0.45	0.85	541	62.79	100.1	100.0
84	T	0.48	0.90	533	70.07	100.0	100.0
83	G	0.44	0.78	467	51.54	100.9	100.0
82	C	0.48	0.79	492	60.85	99.0	100.0
81	C	0.44	0.86	526	61.91	101.7	100.0
80	A	0.44	0.86	483	58.58	99.8	100.0
79	A	0.45	0.85	506	60.23	102.8	100.0
78	C	0.48	0.86	504	61.69	99.9	100.0
77	C	0.49	0.86	508	62.16	100.8	100.0
76	A	0.45	0.84	485	58.36	99.0	100.0
75	C	0.49	0.83	500	61.63	99.6	100.0
74	A	0.49	0.78	488	56.55	98.4	100.0
73	A	0.45	0.85	466	56.36	99.7	100.0
72	T	0.48	0.85	524	65.30	99.4	100.0
71	G	0.48	0.76	444	49.50	99.7	100.0
70	C	0.47	0.85	484	60.90	99.8	100.0
69	A	0.48	0.77	458	55.35	99.5	100.0
68	A	0.49	0.78	486	54.83	99.1	100.0
67	A	0.51	0.81	462	55.51	101.2	100.0
66	C	0.49	0.91	495	61.86	100.4	100.0

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
65	C	0.50	0.84	494	57.78	93.4	100.0
64	A	0.50	0.76	451	52.91	98.4	100.0
63	A	0.50	0.79	463	53.26	100.7	100.0
62	A	0.51	0.85	470	53.82	101.1	100.0
61	A	0.52	0.89	454	53.95	100.2	100.0
60	A	0.47	0.78	638	47.71	88.4	99.9
59	G	0.49	0.76	401	44.63	99.1	99.9
58	A	0.50	0.79	422	51.60	104.0	99.9
57	A	0.51	0.86	436	51.40	99.6	99.9
56	A	0.51	0.79	411	51.72	100.6	99.9
55	C	0.52	0.83	455	54.09	99.3	99.9
54	T	0.51	0.87	455	59.26	99.5	99.9
53	C	0.53	0.90	441	56.49	102.2	100.0
52	C	0.54	0.82	434	53.91	95.4	99.9
51	A	0.52	0.76	388	47.72	98.4	99.9
50	A	0.54	0.83	411	50.51	105.8	100.0
49	A	0.55	0.81	394	48.76	96.5	99.9
48	C	0.52	0.81	425	51.73	99.0	99.9
47	C	0.55	0.81	414	50.53	97.7	99.9
46	C	0.56	0.85	385	50.85	100.6	99.9
45	T	0.55	0.93	426	56.37	99.4	99.9
44	T	0.56	0.95	432	58.19	103.2	99.9
43	A	0.55	0.85	400	47.77	99.7	99.9
42	G	0.56	0.78	353	40.52	99.4	99.9
41	C	0.55	0.90	408	52.46	100.6	99.9
40	A	0.54	0.79	391	46.41	99.0	99.9
39	C	0.56	0.88	386	51.20	98.8	99.9
38	G	0.55	0.84	343	40.82	100.2	99.9
37	A	0.56	0.89	366	47.86	101.0	99.9
36	G	0.56	0.84	328	40.79	100.0	99.9
35	T	0.56	0.97	433	55.67	99.5	99.9
34	A	0.57	0.90	381	47.84	100.0	99.9

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
33	C	0.57	0.93	376	51.03	99.9	99.9
32	C	0.58	0.88	376	51.24	100.4	99.9
31	A	0.57	0.81	373	45.68	98.5	99.9
30	C	0.57	0.94	383	50.50	99.3	99.9
29	G	0.58	0.84	317	39.62	99.6	99.9
28	T	0.59	0.91	387	53.99	99.6	99.9
27	G	0.57	0.80	338	40.06	100.6	99.9
26	C	0.56	0.88	379	49.67	99.6	99.9
25	C	0.58	0.96	372	50.96	102.6	99.9
24	A	0.58	0.86	336	43.44	99.3	99.9
23	G	0.58	0.87	304	38.83	99.2	99.9
22	A	0.57	0.90	359	45.95	102.8	99.9
21	T	0.58	1.00	395	54.37	100.1	99.9
20	G	0.57	0.90	321	41.03	101.9	99.9
19	C	0.59	0.96	371	49.06	99.4	99.9
18	T	0.60	1.00	366	55.07	100.4	99.9
17	C	0.60	0.88	351	49.14	100.1	99.9
16	T	0.58	0.97	389	54.20	99.2	99.9
15	G	0.56	0.85	302	39.83	99.4	99.9
14	G	0.59	0.93	314	41.29	103.7	100.0
13	A	0.60	0.88	326	44.26	99.6	100.0
12	G	0.60	0.83	290	39.13	97.3	99.9
11	G	0.58	0.91	303	39.32	100.5	99.9
10	G	0.60	0.92	294	39.74	101.1	100.0
9	C	0.61	1.00	349	49.91	100.2	100.0
8	C	0.60	0.92	335	46.87	93.9	99.9
7	C	0.61	0.97	349	47.56	101.5	99.9
6	C	0.62	1.00	342	47.92	100.8	99.9
5	G	0.61	0.87	281	37.45	98.8	99.9
4	T	0.62	1.00	342	51.26	99.5	99.9
3	G	0.61	0.85	275	37.73	100.4	99.9

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
2	A	0.62	0.96	298	43.10	99.8	99.9
1	G	0.60	0.97	285	38.77	101.4	99.9

Total synthesis yield from start = 92.2%

TABLE 14

Gene Assembler Plus
Sequence: GB2
Synthesis: GB2
Scale: 0.2 micromole
Sequence Length: 117
Column: 1
Final Detritylation: Yes
Coupling Efficiency Threshold: 50%

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
117	C	0.39	0.75	372	41.62	---	---
116	T	0.38	0.82	476	57.61	---	---
115	C	0.38	0.85	614	73.98	---	---
114	A	0.38	0.83	681	76.89	---	---
113	C	0.37	0.87	650	80.01	104.0	100.0
112	G	0.36	0.82	620	69.75	---	100.0
111	G	0.36	0.82	615	70.81	101.5	100.0
110	G	0.36	0.86	587	70.61	99.7	100.0
109	G	0.40	0.87	584	71.03	100.6	100.0
108	C	0.40	0.93	612	82.94	100.7	100.0
107	C	0.44	0.94	615	82.33	99.3	100.0
106	C	0.41	0.96	596	82.53	100.2	100.0
105	T	0.45	1.00	602	88.44	104.0	100.0
104	C	0.43	0.99	594	84.77	101.3	100.0
103	C	0.46	1.00	565	83.42	98.4	100.0
102	A	0.41	0.96	530	74.96	99.8	100.0
101	G	0.43	0.92	489	68.96	99.6	100.0
100	A	0.45	1.00	536	78.05	102.0	100.0
99	G	0.46	0.96	474	68.87	99.9	100.0
98	C	0.49	1.00	537	81.87	99.6	100.0

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
97	A	0.43	0.96	505	73.90	98.2	100.0
96	T	0.50	1.00	551	87.19	99.8	100.0
95	C	0.51	1.00	528	81.58	99.9	100.0
94	T	0.51	1.00	523	89.78	101.5	100.0
93	G	0.44	0.97	457	67.55	99.7	100.0
92	G	0.55	0.97	453	68.30	101.1	100.0
91	C	0.54	1.00	497	79.14	99.2	100.0
90	A	0.56	1.00	480	74.19	100.1	100.0
89	C	0.55	1.00	496	79.76	100.4	100.0
88	G	0.48	0.99	431	65.21	98.8	100.0
87	T	0.57	1.00	516	83.81	99.0	100.0
86	G	0.48	1.00	430	65.28	100.1	100.0
85	G	0.58	0.94	429	65.09	99.7	100.0
84	T	0.60	1.00	498	83.19	99.8	100.0
83	A	0.59	1.00	462	73.76	99.9	100.0
82	C	0.58	1.00	480	75.66	99.2	100.0
81	T	0.59	1.00	503	83.05	99.9	100.0
80	C	0.58	1.00	472	77.28	101.1	100.0
79	G	0.51	1.00	400	62.70	99.4	100.0
78	T	0.60	1.00	485	82.91	99.9	100.0
77	G	0.52	1.00	409	62.78	100.1	100.0
76	C	0.60	1.00	450	74.44	99.1	100.0
75	T	0.60	1.00	474	80.50	99.0	100.0
74	A	0.61	1.00	431	70.02	99.4	100.0
73	A	0.61	1.00	409	64.69	92.4	99.9
72	G	0.56	0.99	417	58.83	98.7	99.9
71	G	0.63	1.00	392	62.01	105.4	100.0
70	G	0.63	0.97	374	59.64	96.2	99.9
69	T	0.63	1.00	451	76.83	99.2	99.9
68	T	0.64	1.00	456	79.06	102.9	100.0
67	T	0.63	1.00	461	81.55	103.1	100.0
66	G	0.54	1.00	378	61.28	100.7	100.0

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
65	G	0.55	1.00	377	62.27	101.6	100.0
64	A	0.62	1.00	387	64.47	100.0	100.0
63	G	0.56	1.00	362	60.26	98.4	100.0
62	T	0.64	1.00	447	78.76	99.3	100.0
61	T	0.65	1.00	437	79.33	100.7	100.0
60	T	0.63	1.00	454	81.60	102.9	100.0
59	C	0.63	1.00	415	72.64	99.9	100.0
58	T	0.63	1.00	438	78.53	98.1	100.0
57	T	0.60	1.00	442	79.53	101.3	100.0
56	T	0.61	1.00	449	80.46	101.2	100.0
55	T	0.61	1.00	449	80.76	100.4	100.0
54	T	0.61	1.00	465	83.66	103.6	100.0
53	G	0.55	1.00	393	67.17	101.1	100.0
52	G	0.55	1.00	401	68.33	101.7	100.0
51	T	0.63	1.00	428	77.66	97.5	100.0
50	T	0.63	1.00	448	80.73	104.0	100.0
49	T	0.64	1.00	437	79.86	98.9	100.0
48	G	0.59	1.00	375	66.23	99.2	100.0
47	C	0.59	1.00	438	75.14	100.3	100.0
46	A	0.56	1.00	413	71.37	100.6	100.0
45	T	0.58	1.00	430	77.83	99.4	100.0
44	T	0.59	1.00	432	78.71	101.1	100.0
43	G	0.55	1.00	429	73.64	102.1	100.0
42	T	0.59	1.00	442	81.70	101.9	100.0
41	G	0.56	1.00	422	70.65	97.9	100.0
40	G	0.55	1.00	416	71.06	100.6	100.0
39	T	0.59	1.00	434	80.87	99.7	100.0
38	T	0.62	1.00	416	78.10	96.6	100.0
37	G	0.57	0.97	398	69.82	99.4	100.0
36	G	0.56	1.00	390	69.72	99.9	100.0
35	C	0.58	1.00	391	71.25	99.6	100.0
34	A	0.59	0.95	360	64.87	99.2	100.0

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
33	G	0.56	0.93	348	62.91	96.6	100.0
32	C	0.60	1.00	350	67.06	98.0	100.0
31	G	0.57	1.00	337	63.40	100.4	100.0
30	G	0.57	1.00	327	61.10	96.5	100.0
29	G	0.57	1.00	323	61.26	100.1	100.0
28	T	0.62	1.00	335	66.15	98.4	100.0
27	G	0.57	1.00	310	60.34	99.2	100.0
26	T	0.61	1.00	322	66.58	100.3	100.0
25	C	0.62	1.00	310	62.46	99.0	100.0
24	C	0.60	1.00	305	63.62	101.9	100.0
23	T	0.63	1.00	319	65.88	99.6	100.0
22	G	0.59	0.99	298	59.70	99.8	100.0
21	C	0.62	1.00	299	62.66	99.5	100.0
20	A	0.61	0.98	288	58.54	99.3	100.0
19	G	0.58	0.96	281	57.39	98.7	100.0
18	A	0.58	1.00	276	56.57	98.3	100.0
17	T	0.62	1.00	287	61.25	98.8	100.0
16	C	0.60	1.00	277	59.14	98.9	99.9
15	A	0.60	0.98	277	58.47	101.1	100.0
14	A	0.60	0.98	272	57.73	98.7	99.9
13	G	0.56	0.94	261	54.97	99.3	99.9
12	G	0.56	1.00	257	54.71	99.5	99.9
11	G	0.60	1.00	259	54.78	100.1	99.9
10	C	0.60	1.00	258	56.94	99.4	99.9
9	C	0.62	1.00	258	56.08	98.5	99.9
8	C	0.60	1.00	256	57.29	102.2	99.9
7	A	0.61	0.98	253	55.40	99.4	99.9
6	C	0.64	1.00	250	56.66	99.4	99.9
5	C	0.61	1.00	249	56.53	99.8	99.9
4	G	0.58	1.00	242	54.15	99.8	99.9
3	C	0.63	1.00	243	55.26	98.9	99.9

Pos	Base	Retention mins	Duration mins	Peak ht %FS	Acc Area %min	Last eff %	Ave eff %
2	C	0.60	1.00	243	56.87	102.9	99.9
1	A	0.62	1.13	243	56.76	100.4	99.9

Total Synthesis yield from start = 94.3%

Example VIII

The majority of parenterally transmitted non-A, non-B hepatitis (PT-NANBH) cases are believed to be caused by the hepatitis C virus (HCV). See, for example, Choo, Q-L. et al.: Science, 244: 359-362 (1989); Kuo, G. et al.: Science, 244:362-364 (1989); and Choo, Q-L. et al.: Br. Med. Bul., 46:423-441 (1990). Possible sources of infection for a substantial number of remaining cases, known as sporadic hepatitis C, have been controversial. It has been suggested that heterosexual activity, see for example Alter, M.J. et al.: JAMA, 262:1201-1205 (1989); Alter, M.J. et al.: JAMA, 264: 2231-2235 (1990); and Melbye, M. et al.: Br. Med. J., 301:210-212 (1990), and possibly homosexual activity, Tedder, R.S. et al.: Br. Med. J., 302: 1299-1302 (1991), may be important, perhaps accounting for up to an additional 11% of all cases seen. In order to obtain more direct evidence for the potential of sexual transmission of HCV, enzyme-linked immunosorbent assays (ELISA) based upon polyclonal antibodies raised against either the FGB1 or FGB2 polypeptides in New Zealand rabbits were used to detect HCV in semen samples.

The two polypeptide antigens, FGB1 and FGB2, were synthetically constructed as set forth in detail hereinbefore. The FGB1 and FGB2 polypeptides included the cysteine and tyrosine amino acid residues

at their respective $-NH_2$ and -COOH terminal ends. Each antigen was injected into New Zealand rabbits along with Freund's adjuvant using standard protocols, as described in Example III.

In order to determine the titers of the antibodies made, ELISA plates coated separately with about 100 ng of the two antigens were treated with the serial dilutions of the prebleed and the three-month bleeds from the rabbits for 4-6 hr. at room temperature. The plates then were washed five times with phosphate-buffered saline containing about 0.1% Tween and treated with goat anti-rabbit conjugated with horse radish peroxidase (diluted 1:200) for about 2-4 hr. The washed plates were then treated with a substrate solution prepared by dissolving four OPD (1,2-phenylenediamine dihydrochloride) tablets (2mg/tablet) in about 12 ml of about 0.1 M citric acid-phosphate, about pH 5.0, and further adding about 5 micro liters of about 30% H_2O_2. The plates then were incubated in the dark for about 5 min. at about room temperature. To stop the reaction, about 50 micro liters of about 1 M H_2SO_4 were added to each well and absorbance of the orange-colored end-product was read by a microwell plate reader at 450/492 nm. TABLES 11 and 12 show the absorbance values obtained for the serial dilution of the prebleed and the three-month bleed for each of the two antigens, i.e.,

FGB1 and FGB2. The presence of antibodies in a very high dilution (1:1,000,000) suggests that both FGB1 and FGB2 antigens are highly immunogenic and that the antibodies are likely to be very sensitive reagents for the detection of structural components of HCV.

Blood-free semen samples from nine patients with clinical manifestation of NANBH and from five healthy donors were homogenized and treated with about 0.5% NP-40 in phosphate-buffered saline to obtain a final concentration of about 0.1% NP-40 at about room temperature. The contents were centrifuged at about 12,000 rpm for about 15 min. at about 4°C. The plates were then washed five times with PBS containing about 0.1% Tween 20 and treated with the rabbit antibody at a dilution of about 1:100 for about 6-8 hr. at approximately room temperature. The plates were washed once again and treated as described above with goat anti-rabbit antibody and further with the substrate solution; the absorbance values obtained at 450/492 nm are reported in TABLE 15.

TABLE 15

SEMEN ANALYSIS

Patient	FGB2	FGB1
1	0.813	1.519
2	2.052	2.796
3	1.420	2.363
4	1.110	1.930
5	1.910	2.399
6	1.339	2.045
7	1.224	1.622
8	0.854	1.420
9	0.709	1.513
Mean of 5 Control Semen	0.500	0.900
Range of Control Semen	0.380-0.640	0.700-1.200

In order to verify whether patients whose semen contained HCV-specific antigen had HCV-specific antibodies in their serum, we tested the serum from eight of the nine patients. We coated ELISA plates with about 50 ng of either FGB1 or FGB2 and added about 100 micro liters of the eight serum samples from the healthy donors, which were diluted about 1:5 in PBS-Tween to individual wells. The procedure was identical to the one described above except that a mixture of goat anti-human and rabbit anti-human I_gG conjugated with horse radish peroxidase was used as a second antibody in place of goat anti-rabbit. The absorbance values are shown in TABLE 16 under the columns of FGB1 and FGB2. The cutoff values were obtained by multiplying by a factor of 1.5 the mean absorbance value obtained by averaging the absorbance values for the five healthy donors. The serum samples were also tested with the commercially available Ortho test kit, i.e., C-100 nonstructural antigen, and the values are also shown in TABLE 16.

TABLE 16

SERUM ANALYSIS

Patient	FGB2	FGB1	Ortho	Interp.*
Cutoff	0.768	0.220	0.140	R
1	1.042	2.669	2.215	R
2	1.340	0.320	0.300	R
3	3.298	2.545	2.208	R
4	0.633	0.681	0.079	R
5	1.100	0.829	2.262	R
6	2.662	2.713	2.287	R
7	NA	NA	NA	NA
8	1.306	2.761	0.961	R
9	1.112	2.423	0.672	R

*Interp. = Interpretation; R = Reactive;
NA = Not Available

All nine semen samples obtained from NANBH patients had absorbance values higher than the values obtained from the healthy donors employing antibodies to each of the two antigens, FGB1 and FGB2. Significantly higher absorbance values (greater than twice the mean absorbance value for health donors) were obtained in five of the nine semen samples. See TABLE 15.

Available serum from eight patients was reactive. Patient 4 was nonreactive with both the Ortho test and an ELISA using HCV-FGB1, but was repeatedly positive ($A_{492\ nm}$ 1.669 at a cutoff of 0.447) with the ELISA using FGB2 as antigen. Based upon the results, all patients tested are believed to be infected with HCV, and are further believed to

contain varying amounts of HCV viral antigen in their semen.

This study is believed to have detected the presence of HCV-specific viral antigens in the semen of nine patients with well-documented HCV infection. In contrast, semen from five healthy controls did not show HCV antigen presence. These findings support the potential for sexual transmission of hepatitis C virus.

The present invention may, of course, be carried out in other specific ways than those herein set forth without departing from the spirit and essential characteristics of the invention. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive and all changes coming within the meaning and equivalency range of the appended claims are intended to be embraced herein.

APPENDIX I
SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Kotwal, Girish J.
Baroudy, Bahige M.

(ii) TITLE OF INVENTION: Basic Structural Immunogenic Polypeptides having Epitopes for HCV, Antibodies, Polynucleotide Sequences, Vaccines, and Methods

(iii) NUMBER OF SEQUENCES: 75

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: Wood, Herron & Evans
(B) STREET: 2700 Carew Tower
(C) CITY: Cincinnati
(D) STATE: Ohio
(E) COUNTRY: U.S.A.
(F) ZIP: 45202

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE: 14-JAN-1992
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/639,809
(B) FILING DATE: 14-JAN-1991

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Manso, Peter J.
(B) REGISTRATION NUMBER: 32,264

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: (513) 241-2324
(B) TELEFAX: (513) 421-7269
(C) TELEX: 501826

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Cys Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: C-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser Arg
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: hepatitis C virus

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

AAACCUCAAA GAAAAACCAA ACGUAACACC AACCGUCGCC CACAG 45

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..48
(D) OTHER INFORMATION: /function= "Epitope"
/product= "Polypeptide having epitope for HCV"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

UGYAAACCUC AAAGAAAAAC CAAACGUAAC ACCAACCGUC GCCCACAG 48

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..48
(D) OTHER INFORMATION: /function= "Epitope"
/product= "Polypeptide having epitope for HCV"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

AAACCUCAAA GAAAAACCAA ACGUAACACC AACCGUCGCC CACAGUAY 48

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: misc_RNA
(B) LOCATION: 1..51
(D) OTHER INFORMATION: /function= "Epitope"
/product= "Polypeptide having epitope for HCV"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

UGYAAACCUC AAAGAAAAAC CAAACGUAAC ACCAACCGUC GCCCACAGUA Y 51

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

AAACCTCAAA GAAAAACCAA ACGTAACACC AACCGTCGCC CACAG 45

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

TGYAAACCTC AAAGAAAAAC CAAACGTAAC ACCAACCGTC GCCCACAG 48

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

AAACCTCAAA GAAAAACCAA ACGTAACACC AACCGTCGCC CACAGTAY 48

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

TGYAAACCTC AAAGAAAAAC CAAACGTAAC ACCAACCGTC GCCCACAGTA Y 51

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

UCUCGGCCUA GUUGGGGCCC CAGGGACCCC CGGCGUAGGU CGCGC 45

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

UGYUCUCGGC CUAGUUGGGG CCCCAGGGAC CCCCGGCGUA GGUCGCGC 48

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

UCUCGGCCUA GUUGGGGCCC CAGGGACCCC CGGCGUAGGU CGCGCUAY 48

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

UGYUCUCGGC CUAGUUGGGG CCCCAGGGAC CCCCGGCGUA GGUCGCGCUA Y 51

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

TCTCGGCCTA GTTGGGGCCC CAGGGACCCC CGGCGTAGGT CGCGC 45

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

TGYTCTCGGC CTAGTTGGGG CCCCAGGGAC CCCCGGCGTA GGTCGCGC 48

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 48 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

TCTCGGCCTA GTTGGGGCCC CAGGGACCCC CGGCGTAGGT CGCGCTAY 48

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 51 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

TGYTCTCGGC CTAGTTGGGG CCCCAGGGAC CCCCGGCGTA GGTCGCGCTA Y 51

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

UUUGGAGUUU CUUUUUGGUU UGCAUUCUGG UUGGCAGCGG GUGUC 45

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

TTTGGAGTTT CTTTTTGGTT TGCATTCTGG TTGGCAGCGG GTGTC 45

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: RNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

AGAGCCGGAU CAACCCCGGG GUCCCUGGGG GCCGCAUCCA GCGCG 45

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

AGAGCCGGAT CAACCCCGGG GTCCCTGGGG GCCGCATCCA GCGCG 45

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

GACACCCGCT GCCAACCACA ATGCAAACCA AAAAGAAACT CCAAA 45

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 45 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

CGCGCTGGAT GCGGCCCCCA GGGACCCCGG GGTTGATCCG GCTCT 45

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: insertion_seq
(B) LOCATION: 1..117

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 34..102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

```
GAGTGCCCCG GGAGGTCTCG TAGACCGTGC ACC ATG AGC ACG ATT CCC AAA CCT 54
                                      Met Ser Thr Ile Pro Lys Pro
                                        1               5

CAA AGA AAA ACC AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC      99
Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val
         10                  15                  20

TAGTTCCCGG GTGGCGGT                                            117
```

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
  1               5                  10                  15
Arg Arg Pro Gln Asp Val
             20
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 117 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: insertion_seq
(B) LOCATION: 1..117

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 34..102

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
GAGTGCCCC GGGAGGTCTC GTAGACCGTG CACC ATG AGC ACG ATT CCC AAA    51
                                      Met Ser Thr Ile Pro Lys
                                        1               5

CCT CAA AGA AAA ACC AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC 99
Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val
            10                  15                  20

TAGTTCCCG GGTGGCGGT                                            117
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

```
Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
  1               5                  10                  15

Arg Arg Pro Gln Asp Val
            20
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: insertion_seq
(B) LOCATION: 1..98

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 25..93

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
GGGAGGTCTC GTAGACCGTG CACC ATG AGC ACG ATT CCC AAA CCT CAA AGA AAA 54
                           Met Ser Thr Ile Pro Lys Pro Gln Arg Lys
                             1               5                  10

ACC AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC TAGTTCCC          98
Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val
                 15                  20
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
1               5                  10                  15

    Arg Arg Pro Gln Asp Val
                20
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 98 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: insertion_seq
(B) LOCATION: 1..98

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 25..93

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

GGGAGGTCTC GTAGACCGTK CACCATGAGC ACGATTCCCA AACCTCAAAG AAAAACCAAA 60

CGTAAMACCA ACCGTCGCCC ACAGGACGTC TAGTTCCC 98

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
1 5 10 15

Arg Arg Pro Gln Asp Val
20

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Lys Thr Asn
1 5 10 15

Arg Arg Pro Gln Asp Val
20

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Polypeptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Cys Arg Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Cys Lys Pro Gln Arg Arg Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Cys Lys Pro Gln Arg Lys Thr Arg Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Cys Lys Pro Gln Lys Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Cys Lys Pro Gln Arg Lys Thr Lys Lys Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Lys Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Lys Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Cys Lys Pro Gln Arg Lys Ser Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Ser Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Cys Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Cys Lys Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

Cys Arg Arg Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

Cys Arg Lys Thr Arg Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

Cys Arg Lys Ser Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

Cys Arg Lys Thr Lys Lys Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

Cys Arg Lys Thr Lys Arg Asn Thr Asn Lys Arg Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Cys Arg Lys Thr Lys Arg Asn Thr Asn Arg Lys Pro Gln Tyr
1 5 10

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

Cys Lys Gln Lys Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58

Lys Gln Lys Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

Lys Pro Gln Lys Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

Lys Pro Gln Lys Lys Asn Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

Cys Lys Gln Arg Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

Lys Gln Arg Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:63:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

Cys Lys Gln Arg Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptid

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

Lys Pro Gln Arg Lys Thr Lys Arg Ser Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

Cys Lys Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..13
(D) OTHER INFORMATION: /label= Peptide
/note= "P lypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

Lys Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

Lys Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg
1 5 10

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

Lys Pro Gln Arg Lys Asn Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..16
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

Cys Lys Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Tyr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..14
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

Lys Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

Cys Lys Pro Gln Lys Lys Asn Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

Cys Lys Pro Gln Arg Lys Asn Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..17
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

Cys Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Tyr

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal

(ix) FEATURE:
(A) NAME/KEY: Peptide
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /label= Peptide
/note= "Polypeptide having epitope for HCV."

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

Lys Pro Gln Lys Lys Asn Lys Arg Asn Thr Asn Arg Arg Pro Gln
1 5 10 15

Having described our invention, we claim:

1. A basic, immunogenic polypeptide having an epitope for HCV, said basic, immunogenic polypeptide further having at least about 15 amino acid residues in its sequence of which at least about 4 said residues are arginine amino acid residues and at least about 7 said residues are in a Chou-Fasman turn.

2. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having between about 15 and about 17 amino acid residues in its sequence.

3. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having between about 7 and about 17 amino acid residues in a Chou-Fasman turn.

4. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having a calculated molecular weight of at least about 1500.

5. A basic, immunogenic polypeptide as recited in claim 4, said basic, immunogenic polypeptide further having a calculated molecular weight of between about 1500 and about 2500.

6. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having a pI on the order of about 12.

7. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having between about 4 and about 7 arginine amino acid residues in its sequence.

8. A basic, immunogenic polypeptide as recited in claim 7, said basic, immunogenic polypeptide further having about 4 arginine amino acid residues and up to about 3 lysine amino acid residues in its sequence.

9. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having at least about 11 hydrophillic amino acid residues in its sequence.

10. A basic, immunogenic polypeptide as recited in claim 9, said basic, immunogenic polypeptide further having between about 11 and about 14 hydrophillic amino acid residues in its sequence.

11. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having at least about 5 polar amino acid residues in its sequence.

12. A basic, immunogenic polypeptide as recited in claim 11, said basic, immunogenic polypeptide further having between about 5 and about 8 polar amino acid residues in its sequence.

13. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having at least about 5 basic amino acid residues in its sequence.

14. A basic, immunogenic polypeptide as recited in claim 13, said basic, immunogenic polypeptide further having between about 5 and about 7 basic amino acid residues in its sequence.

15. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having an amino acid sequence which corresponds to an amino acid sequence encoded by a polynucleotide sequence that lies within the first 4% of a human HCV genome following an initiation codon encoding for a human HCV polyprotein.

16. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide further having an amino acid sequence which corresponds to an amino acid sequence encoded by a polynucleotide sequence in the structural region of a human HCV genome.

17. A polypeptide comprised of said basic, immunogenic polypeptide as recited in claim 1.

18. A basic, immunogenic polypeptide as recited in claim 1, said basic, immunogenic polypeptide being attached to a solid substrate.

19. A truncated, basic immunogenic polypeptide having an epitope for HCV, said truncated basic, immunogenic polypeptide further having an amino acid sequence which corresponds to an amino acid sequence encoded by a polynucleotide sequence that lies within the first 4% of a HCV genome following an initiation codon encoding for a human HCV polyprotein.

20. A truncated, basic immunogenic polypeptide as recited in claim 19, said truncated, basic, immunogenic polypeptide amino acid sequence corresponding to an amino acid sequence which is encoded by a polynucleotide sequence in the structural region of a HCV genome.

21. FGB1.

22. An immunogenic polypeptide comprising a carrier and said FGB1 as recited in claim 21.

23. FGB1 as recited in claim 22, said carrier being a polypeptide.

24. FGB1 as recited in claim 21, said FGB1 having a labeling moiety.

25. FGB1 as recited in claim 21, said FGB1 further having the following amino acid sequence

Lys-Pro-Gln-Arg-Lys-Thr-Lys-
Arg-Asn-Thr-Asn-Arg-Arg-Pro-
Gln-Ser-Arg-Pro-Ser-Trp-Gly-
Pro-Thr-Asp-Pro-Arg-Arg-Arg-
Ser-Arg.

26. FGB1 as recited in claim 21, said FGB1 further having the following amino acid sequence

Ser-Arg-Pro-Ser-Trp-Gly-Pro-
Thr-Asp-Pro-Arg-Arg-Arg-Ser-
Arg-Lys-Pro-Gln-Arg-Lys-Thr-
Lys-Arg-Asn-Thr-Asn-Arg-Arg-
Pro-Gln.

27. FGB1 as recited in claim 21, said FGB1 further having the following structure:

NH_2-FGB1-COOH.

28. A polypeptide comprised of said FGB1 as recited in claim 21.

29. A polypeptide as recited in claim 28, said FGB1 being FGB1-Tyr.

30. FGB1 as recited in claim 21, said FGB1 being attached to a substrate.

31. FGB2.

32. An immunogenic polypeptide comprising a carrier and said FGB2 as recited in claim 31.

33. FGB2 as recited in claim 32, said carrier being a polypeptide.

34. FGB2 as recited in claim 31, said FGB2 having a labeling moiety.

35. FGB2 as recited in claim 34, said FGB2 further having the following structure:

NH_2-FGB2-COOH.

36. A polypeptide comprised of said FGB2 as recited in claim 31.

37. A polypeptide as recited in claim 36, said FGB2 being FGB2-Tyr.

38. FGB2 as recited in claim 31, said FGB2 being attached to a substrate.

39. A basic, immunogenic polypeptide having an epitope for HCV, said basic polypeptide further having:

a) at least about 15 amino acids in its sequence;

b) at least about 7 amino acids in a Chou-Fasman turn;

c) at least about 11 hydrophillic amino acid residues in its sequence;

d) at least about 5 polar amino acid residues in its sequence;

e) at least about 5 basic amino acid residues in its sequence; and

f) a calculated molecular weight of at least about 1500.

40. A basic, immunogenic polypeptide as recited in claim 39, said basic, immunogenic polypeptide further having:

a.) between about 15 and 17 amino acid residues;

b.) between about 7 and 17 amino acid residues in a Chou-Fasman turn;

c.) between about 11 and 14 hydrophillic amino acid residues;

d.) between about 5 and 8 polar amino acid residues;

e.) between about 5 and 7 basic amino acid residues;

f.) a calculated molecular weight of between about 1500 and 2500; and

g.) a pI on the order of about 12.

41. A polypeptide comprised of said basic, immunogenic polypeptide as recited in claim 40.

42. A basic, immunogenic polypeptide as recited in claim 40, said basic, immunogenic polypeptide being attached to a substrate.

43. An isolated antibody against FGB1.

44. An isolated antibody as recited in claim 43, said antibody being selected from the group consisting of monoclonal and polyclonal antibodies.

45. An isolated anti-idiotype antibody against said antibody as recited in claim 43.

46. An isolated antibody as recited in claim 43, said FGB1 further comprising a carrier.

47. An isolated antibody as recited in claim 46, said antibody being selected from the group consisting of polyclonal and monoclonal antibodies.

48. An isolated anti-idiotype antibody against said antibody as recited in claim 46.

49. An isolated antibody as recited in claim 46, said antibody having a labeling moiety.

50. An isolated antibody as recited in claim 43, said FGB1 further comprising a polypeptide.

51. An isolated antibody as recited in claim 46, said antibody being attached to a substrate.

52. An isolated antibody as recited in claim 43, said antibody having a labeling moiety.

53. An isolated antibody against FGB2.

54. An isolated antibody as recited in claim 53, said antibody being selected from the group consisting of monoclonal and polyclonal antibodies.

55. An isolated anti-idiotype antibody against said antibody as recited in claim 53.

56. An isolated antibody as recited in claim 53, said FGB2 further comprising a carrier.

57. An isolated antibody as recited in claim 56, said antibody being selected from the group consisting of polyclonal and monoclonal antibodies.

58. An isolated anti-idiotype antibody against said antibody as recited in claim 56.

59. An isolated antibody as recited in claim 56, said antibody having a labeling moiety.

60. An isolated antibody as recited in claim 53, said FGB2 further comprising a polypeptide.

61. An isolated antibody as recited in claim 56, said antibody being attached to a substrate.

62. An isolated antibody as recited in claim 53, said antibody having a labeling moiety.

63. An isolated antibody against a basic, immunogenic polypeptide as recited in claim 1.

64. An isolated antibody against a truncated, basic immunogenic polypeptide as recited in claim 17.

65. An isolated antibody against a truncated, basic immunogenic polypeptide as recited in claim 19.

66. An isolated antibody against a truncated, basic immunogenic polypeptide as recited in claim 20.

67. An isolated antibody against a basic, immunogenic polypeptide as recited in claim 39.

68. An isolated antibody against a basic, immunogenic polypeptide as recited in claim 40.

69. An isolated antibody against a basic, immunogenic polypeptide as recited in claim 41.

70. A polynucleotide sequence comprising

AAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAG.
(U) (U) (U)

71. A probe having at least about 6 nucleotides derived from said polynucleotide sequence as recited in claim 70 or a complement thereto, said probe further having the ability to bind to HCV polynucleotides in a target region.

72. A polynucleotide sequence as recited in claim 70, said polynucleotide sequence further having the following sequence

(TGT)AAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAG(TAT)
(UGU) (U) (U) (U) (UAU)
(TGC) (TAC)
(UGC) (UAC).

73. An antisense polynucleotide sequence comprised of nucleotides which are complementary to said polynucleotide sequence as recited in claim 70, said antisense polynucleotide sequence having the ability to bind to HCV polynucleotides.

74. An antisense polynucleotide as recited in claim 73, said antisense polynucleotide having the following sequence

TTTGGAGTTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGTGTC.
(UUU) (UUU UUUUU)(UUU) (UU U)(UU) (U U)

75. A polynucleotide sequence comprising

TCTCGGCCTAGTTGGGGCCCCAGGGACCCCCGGCGTAGGTCGCGC.
(U U) (U)(UU) (U) (U)

76. A probe having at least about 6 nucleotides derived from said polynucleotide sequence as recited in claim 75 or a complement thereto, said probe further having the ability to bind to HCV polynucleotides in a target region.

77. A polynucleotide sequence as recited in claim 75, said polynucleotide sequence further having the following sequence

(TGT) TCTCGGCCTAGTTGGGGCCCCAGGGACCCCCGGCGTAGGTCGCGCTAT (TAT)
(UGU)(U U) (U)(UU) (U) (U) (U U)(UAU)
(TGC) (TAC)
(UGC) (UAC).

78) An antisense polynucleotide sequence comprised of nucleotides which are complementary to said polynucleotide sequence as recited in claim 75, said antisense polynucleotide sequence having the ability to bind to HCV polynucleotides.

79. An antisense polynucleotide sequence as recited in claim 78, said antisense polynucleotide sequence having the following sequence

AGAGCCGGATCAACCCCGGGGTCCCTGGGGGCCGCATCCAGCGCG.
(U) (U) (U) (U)

80. A double stranded DNA fragment having a polynucleotide sequence selected from the group consisting of

GACACCCGCTGCCAACCACAATGCAAACCAAAAAGAAACTCCAAA
CTGTGGGCGACGGTTGGTGTTACGTTTGGTTTTTCTTTGAGGTTT and

CGCGCTGGATGCGGCCCCCAGGGACCCCGGGGTTGATCCGGCTCT
GCGCGACCTACGCCGGGGGTCCCTGGGGCCCCAACTAGGCCGAGA.

81. A recombinant expression system containing an in frame sequence operably linked to a control sequence compatible with a desired host, said in frame sequence including said DNA fragment as recited in claim 80.

82. A host transformed with said recombinant expression system as recited in claim 81.

83. An antisense polynucleotide strand expressed by said transformant as recited in claim 82.

84.) A double stranded DNA fragment having the following sequence

```
                    (T)
GGGAGGTCTCGTAGACCGTGCACCATGAGCACGATTCCCAAACCTC
CCCTCCAGAGCATCTGGCACGTGGTACTCGTGCTAAGGGTTTGGAG
                    (A)

                    (A)
AAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAGGACGTCTA
TTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGTGTCCTGCAGAT
                    (T)

GTTCCC
CAAGGG.
```

85. A double stranded DNA fragment as recited in claim 84, said DNA fragment further having the following sequence

```
                              (T)
GAGTGCCCCGGGAGGTCTCGTAGACCGTGCACCATGAGCACGATTCCCAAACCT
CTCACGGGGCCCTCCAGAGCATCTGGCACGTGGTACTCGTGCTAAGGGTTTGGA
                              (A)

                    (A)
CAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAGGACGTCTA
GTTTCTTTTTGGTTTGCATTGTGGTTGGCAGCGGGTGTCCTGCAGAT
                    (T)

GTTCCCGGGTGGCGGT
CAAGGGCCCACCGCCA
```

86. A recombinant expression system containing an open reading frame operably linked to a control sequence compatible with a desired host, said open reading frame including said DNA fragment as recited in claim 84.

87. A recombinant expression system comprising a pGEM-3Zf(-) plasmid and said DNA fragment as recited in claim 84.

88. A host transformed with said recombinant expression system as recited in claim 86.

89. A recombinant polypeptide expressed by said transformant as recited in claim 88.

90. FGB1 clone.

91. A kit for analyzing a sample for the presence of HCV polynucleotides, said kit comprising a polynucleotide probe as recited in claim 71, said polynucleotide probe being in a suitable carrier.

92. A kit for analyzing a sample for the presence of HCV polynucleotides, said kit comprising a polynucleotide probe as recited in claim 76, said polynucleotide probe being in a suitable carrier.

93. A kit for analyzing a sample for the presence of an HCV antibody, said kit comprising a polypeptide selected from the group consisting of FGB1 and FGB2 polypeptides, said polypeptide being present in a suitable container.

94. A kit as recited in claim 93, said kit further including a labeling moiety for said polypeptide.

95. A kit as recited in claim 93, said polypeptide further comprising said FGB1 or FGB2 polypeptide and a second polypeptide.

96. A kit as recited in claim 95, said kit further including a labeling moiety for said polypeptide.

97. A kit for analyzing a sample for the presence of HCV antigen, said kit comprising an antibody raised against a polypeptide selected from the group consisting of FGB1 and FGB2 polypeptides.

98. A kit as recited in claim 97, said polypeptide further comprising a carrier.

99. A kit as recited in claim 97, said polypeptide further comprising a second polypeptide.

100. A kit as recited in claim 97, said kit further including a labeling moiety for said antibody.

101. A kit as recited in claim 98, said kit further including a labeling moiety for said antibody.

102. A kit as recited in claim 99, said kit further including a labeling moiety for said antibody.

103. A kit for analyzing a sample for the presence of HCV antibody, said kit comprising an anti-idiotype antibody raised against an antibody as recited in claim 63.

104. A kit for analyzing a sample for the presence of HCV antibody, said kit comprising an anti-idiotype antibody raised against the antibody as recited in claim 17.

105. A kit for analyzing a sample for the presence of HCV antibody, said kit comprising an anti-idiotype antibody raised against said antibody as recited in claim 20.

106. A kit for analyzing a sample for the presence of HCV antibody, said kit comprising an anti-idiotype antibody raised against said antibody as recited in claim 41.

107. A method for detecting HCV nucleotides in a sample suspected of containing HCV, said method comprising:

a.) reacting nucleotides of the sample with the probe as recited in claim 71 under conditions which permit the nucleotides and the probe to form a polynucleotide duplex; and

b.) detecting the formed polynucleotide duplex.

108. A method for detecting HCV nucleotides in a sample suspected of containing HCV, said method comprising:

a.) reacting nucleotides of the sample with the probe as recited in claim 76 under conditions which permit the nucleotides and the probe to form a polynucleotide duplex; and

b.) detecting the formed polynucleotide duplex.

109. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 43 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

110. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 46 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

111. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 50 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

112. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 53 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

113. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 56 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

114. An immunoassay for detecting HCV antigen, said immunoassay comprising:

a.) incubating a sample suspected of containing HCV antigen with an antibody as recited in claim 60 under conditions which permit the HCV antigen and the antibody to form a complex; and

b.) detecting the formed complex.

115. An immunoassay for detecting an antibody directed against HCV, said method comprising:

a.) incubating a sample suspected of containing an anti-HCV antibody with a polypeptide as recited in claim 1 under conditions which permit the anti-HCV antibody and the polypeptide to form a complex; and

b.) detecting the formed antibody-antigen complex.

116. An immunoassay as recited in claim 115, the polypeptide being selected from the group consisting of FGB1 and FGB2 polypeptides.

117. An immunoassay as recited in claim 115, the sample being a blood product.

118. An immunoassay as recited in claim 116, the sample being a blood product.

119. An immunoassay for diagnosing autoimmune liver disease, said method comprising:

a.) incubating a sample derived from a patient suspected of having autoimmune liver disease with an antibody as recited in claim 63 under conditions which allow the antibody to form a complex with an antigen in the sample; and

b.) detecting the formed complex to diagnose autoimmune liver disease.

120. An immunoassay for diagnosing autoimmune liver disease, said method comprising:

a.) incubating a sample derived from a patient suspected of having autoimmune liver disease with an antibody as recited in claim 17 under conditions which allow the antibody to form a complex with an antigen in the sample; and

b.) detecting the formed complex to diagnose autoimmune liver disease.

121. An immunoassay for diagnosing autoimmune liver disease, said method comprising:

a.) incubating a sample derived from a patient suspected of having autoimmune liver disease with an antibody as recited in claim 46 under conditions which allow the antibody to form a complex with an antigen in the sample; and

b.) detecting the formed complex to diagnose autoimmune liver disease.

122. An immunoassay for diagnosing autoimmune liver disease, said method comprising:

a.) incubating a sample derived from a patient suspected of having autoimmune liver disease with an antibody as recited in claim 53 under conditions which allow the antibody to form a complex with an antigen in the sample; and

b.) detecting the formed complex to diagnose autoimmune liver disease.

123. A vaccine for immunizing against HCV infection comprising

a pharmacologically effective dose of a polypeptide selected from the group consisting of FGB1 and FGB2 polypeptides, and

a pharmaceutically acceptable excipient.

124. A vaccine as recited in claim 123, said polypeptide comprising a second polypeptide.

125. A vaccine as recited in claim 123, said polypeptide comprising a carrier.

126. A vaccine as recited in claim 123, said vaccine further including a pharmacologically effective dose of an adjuvant.

127. A vaccine as recited in claim 124, said vaccine further including a pharmacologically effective dose of an adjuvant.

128. A vaccine as recited in claim 125, said vaccine further including a pharmacologically effective dose of an adjuvant.

129. A vaccine for immunizing against HCV infection comprising

a pharmacologically effective dose of a polypeptide selected from the group consisting of a polypeptide as recited in claim 1, a polypeptide comprised of the polypeptide as recited in claim 1 and a polypeptide comprised of the polypeptide as recited in claim 1 and a carrier, and

a pharmaceutically acceptable excipient.

130. A vaccine as recited in claim 129, said vaccine further including a pharmacologically effective dose of an adjuvant.

131. A method of immunizing against HCV infection, said method comprising

administering to an individual a vaccine as recited in claim 129.

132. A method of immunizing against HCV infection, said method comprising

administering to an individual a vaccine as recited in claim 130.

133. A method of producing a recombinant, basic immunogenic polypeptide having an epitope for HCV, said method comprising:

a.) providing a host transformed with a recombinant expression system comprising a coding sequence containing a DNA fragment encoding the polypeptide as recited in claim 1, said coding sequence being operably linked to a control sequence compatible with the host, and

b.) incubating the host under conditions which allow expression of the recombinant, basic immunogenic polypeptide.

134. A method as recited in claim 133 wherein the DNA fragment encodes a polypeptide selected from the group consisting of said polypeptide and a second polypeptide and said polypeptide and a carrier.

135. A method as recited in claim 133 wherein the polypeptide is selected from the group consisting of FGB1 and FGB2 polypeptides.

136. A recombinant expression system comprising a coding sequence including a DNA fragment which encodes the polypeptide as recited in claim 1, said coding sequence being operably linked to a control sequence campatible with a desired host.

137. A host transformed with said recombinant expression system as recited in claim 135.

138. A recombinant expression system as recited in claim 136, said DNA fragment encoding a polypeptide selected from the group consisting of said polypeptide and a second polypeptide, and said polypeptide and a carrier.

139. A recombinant expression system as recited in claim 136, said DNA fragment being selected from the group consisting of

AAACCTCAAAGAAAAACCAAACGTAACACCAACCGTCGCCCACAG
(U) (U) (U)

and

TCTCGGCCTAGTTGGGGCCCCAGGGACCCCCGGCGTAGGTCGCGC.
(U U) (U) (UU) (U) (U)

140. A recombinant expression system as recited in claim 136, said control sequence being derived from a vaccinia virus.

141. A recombinant expression system as recited in claim 136, said polypeptide being selected from the group consisting of FGB1 and FGB2 polypeptides.

142. A recombinant expression system as recited in claim 140, said polypeptide being selected from the group consisting of FGB1 and FGB2 polypeptides.

143. A transformed host as recited in claim 137, said host being a vaccinia virus.

144. A transformed host as recited in claim 137, said control sequence being derived from a vaccinia virus and said polypeptide being selected from the group consisting of FGB1 and FGB2 polypeptides.

145. An immunoassay for detecting an HCV antigen in semen comprising:

a.) incubating semen suspected of containing an HCV antigen with an antibody directed against an HCV epitope wherein said incubation is under conditions which permit the HCV antigen and the antibody to form an antigen-antibody complex; and

b.) detecting the formed complex.

146. An immunoassay as recited in claim 145, said antibody being selected from a group consisting of polyclonal and monoclonal antibodies.

147. An immunoassay as recited in claim 145 including the further step of

raising the antibody against an HCV antigen in an animal.

148. An immunoassay as recited in claim 147, said animal being a rabbit.

149. An immunoassay as recited in claim 145 including the further step of

labeling the antibody with a labeling moiety.

150. An immunoassay as recited in claim 145 including the further step of

attaching a substrate to the antibody.

151. An immunoassay as recited in claim 147, said HCV antigen being a basic immunogenic polypeptide having an epitope for HCV.

152. An immunoassay as recited in claim 151, said basic, immunogenic HCV polypeptide being FGB1.

153. An immunoassay as recited in claim 151, said basic, immunogenic HCV polypeptide being FGB2.

154. An immunoassay as recited in claim 151, said basic, immunogenic HCV polypeptide being selected from the group consisting of FGB3, FGB4, FGB5, FGB6, FGB7, FGB8, FGB9, FGB10, FGB11, FGB12, FGB13, FGB14, FGB15, FGB16, FGB17, FGB18, FGB19, FGB20, FGB21, FGB22, FGB23, FGB24, FGB25, FGB26, FGB27, FGB28, FGB29, FGB30, FGB31, FGB32, FGB33, FGB34, FGB35, FGB36, FGB37 and FGB38.

155. An immunoassay as recited in claim 145 including the further step of

attaching the antibody to a solid substrate.

156. An immunoassay for detecting a passive antibody directed against an HCV antigen comprising:

a.) incubating a sample suspected of containing a passive anti-HCV antibody with an HCV antigen under conditions which permit the passive anti-HCV antibody and the HCV antigen to form a complex; and

b.) detecting the formed passive anti-HCV antibody-HCV antigen complex.

157. An immunoassay as recited in claim 156, said HCV antigen being a basic, immunogenic polypeptide having an epitope for HCV.

158. An immunoassay as recited in claim 157, said basic, immunogenic HCV polypeptide being FGB1.

159. An immunoassay as recited in claim 157, said basic, immunogenic HCV polypeptide being selected from the group consists of FGB2, FGB3, FGB4, FGB5, FGB6, FGB7, FGB8, FGB9, FGB10, FGB11, FGB12, FGB13, FGB14, FGB15, FGB16, FGB17, FGB18, FGB19, FGB20, FGB21, FGB22, FGB23, FGB24, FGB25, FGB26, FGB27, FGB28, FGB29, FGB30, FGB31, FGB32, FGB33, FGB34, FGB35, FGB36, FGB37 and FGB38.

160. An immunoassay as recited in claim 157 including the further step of labeling the polypeptide.

161. An immunoassay as recited in claim 157 including the further step of fusing the polypeptide to a second polypeptide.

162. An immunogenic assay as recited in claim 157, said polypeptide including a labeling moiety.

163. An immunogenic assay as recited in claim 157, said polypeptide including a carrier.

164. An immunogenic assay as recited in claim 156 including the further step of attaching the HCV antigen to a solid substrate.

165. An immunogenic assay as recited in claim 156, said sample being a blood product.

166. A basic, immunogenic polypeptide having an epitope for HCV, said polypeptide being selected from a group consisting of FGB3, FGB4, FGB5, FGB6, FGB7, FGB8, FGB9, FGB10, FGB11, FGB12, FGB13, FGB14, FGB15, FGB16, FGB17, FGB18, FGB19, FGB20, FGB21, FGB22, FGB23, FGB24, FGB25, FGB26, FGB27, FGB28, FGB29, FGB30, FGB31, FGB32, FGB33, FGB34, FGB35, FGB36, FGB37 and FGB38.

167. A basic, immunogenic HCV polypeptide as recited in claim 166, said HCV polypeptide further including a carrier.

168. A basic, immunogenic HCV polypeptide as recited in claim 166, said HCV polypeptide further including a labeling moiety.

169. A basic, immunogenic HCV polypeptide as recited in claim 166, said HCV polypeptide being fused to a second polypeptide.

170. A basic immunogenic HCV polypeptide as recited in claim 166, said HCV polypeptide being attached to a solid substrate.

171. A basic immunogenic HCV polypeptide as recited in claim 168, said HCV polypeptide being attached to a solid substrate.

172. A basic immunogenic HCV polypeptide as recited in claim 169, said HCV polypeptide being attached to a solid substrate.

FIG.1

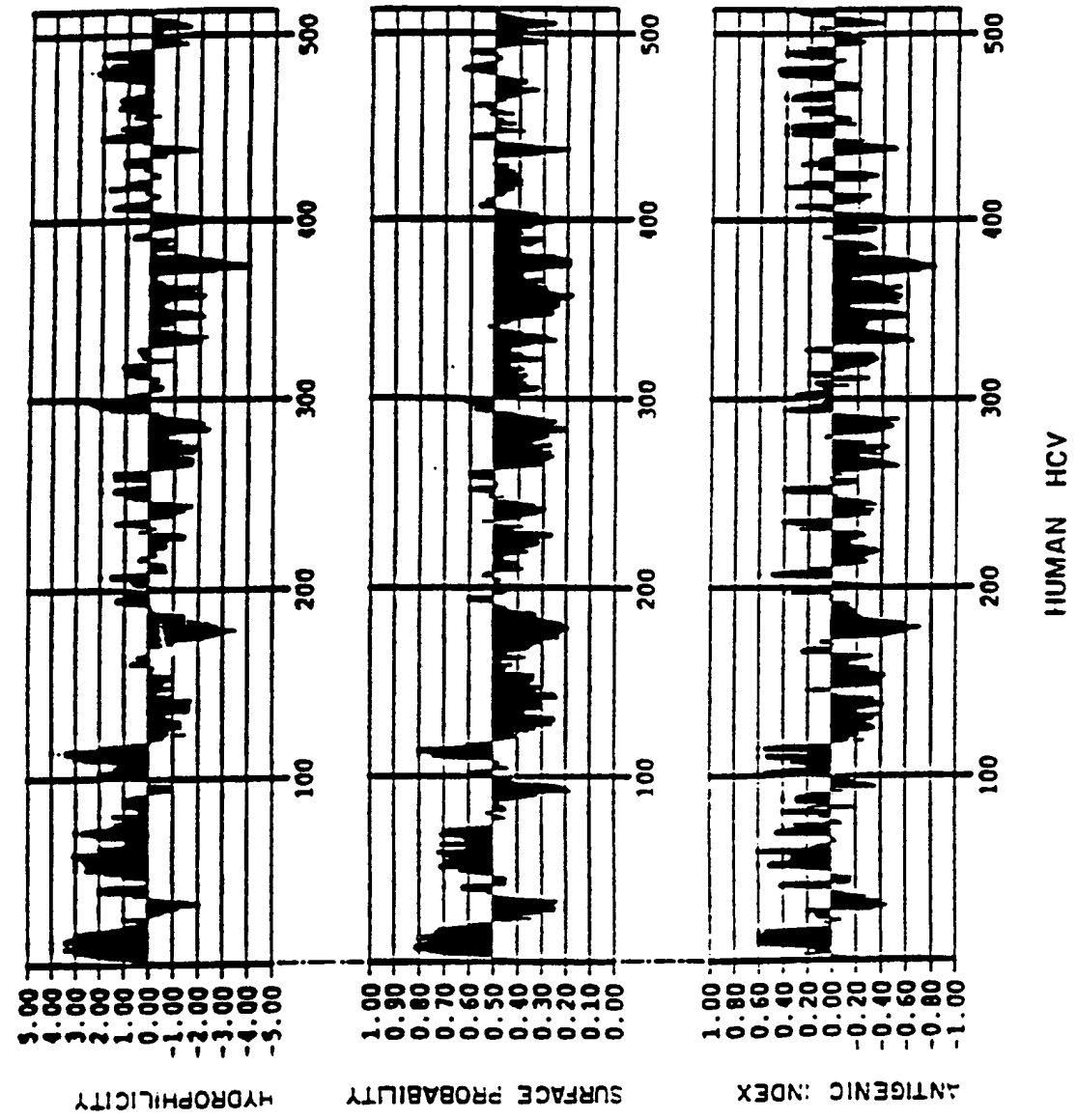
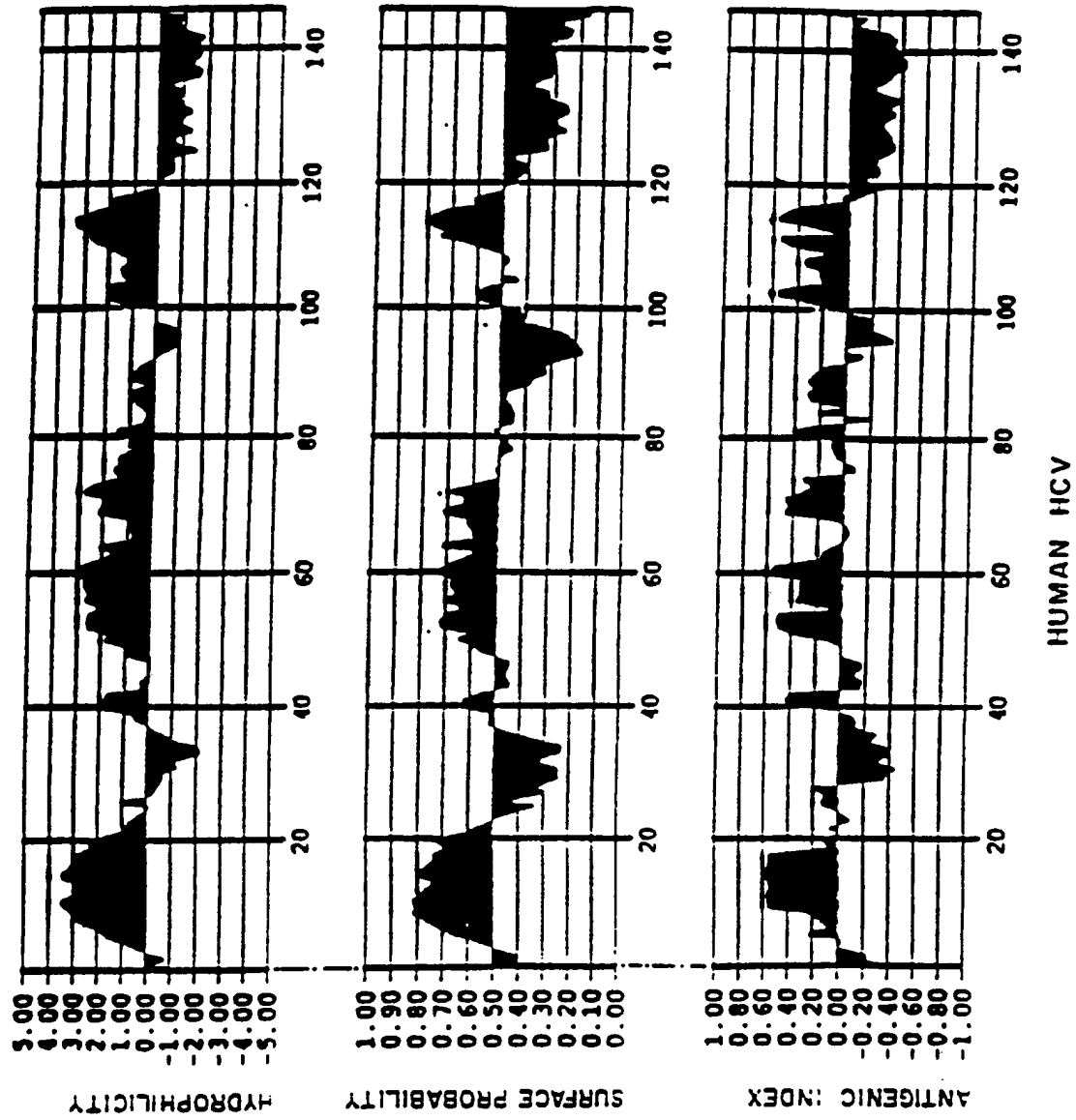
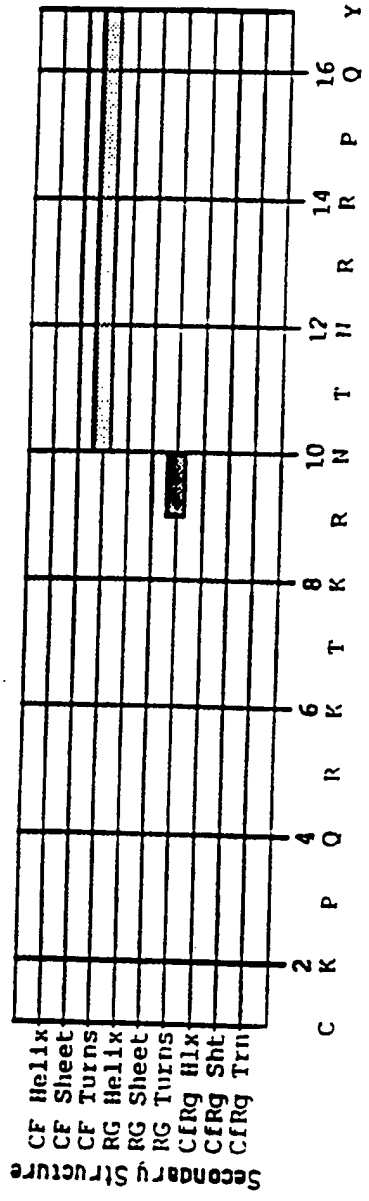

HUMAN HCV
ANTIGENIC INDEX
SURFACE PROBABILITY
HYDROPHILICITY
100
200
300
400
500

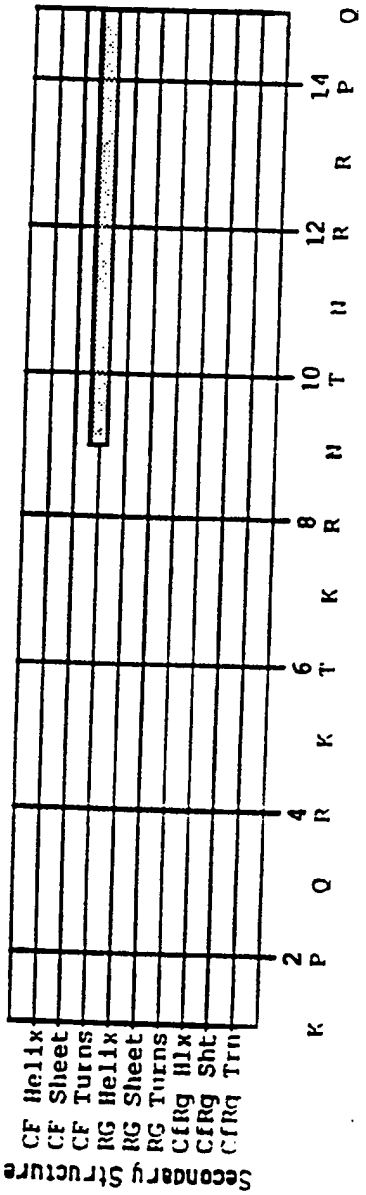
FIG.2


HUMAN HCV
ANTIGENIC INDEX
SURFACE PROBABILITY
HYDROPHILICITY



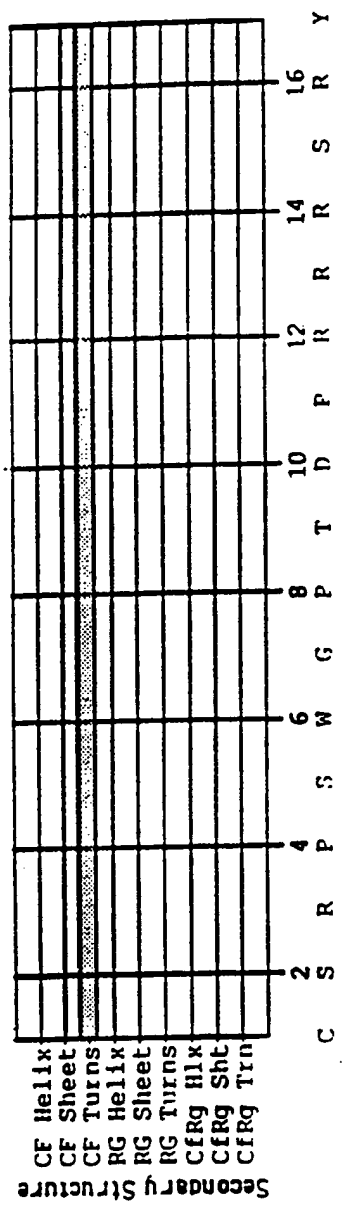
Cys-FGBI-Tyr

FIG.3



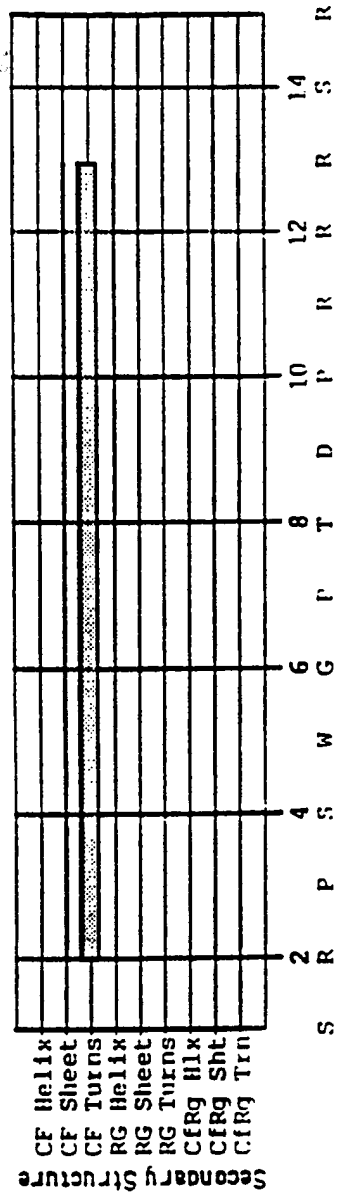
FGBI

FIG.4



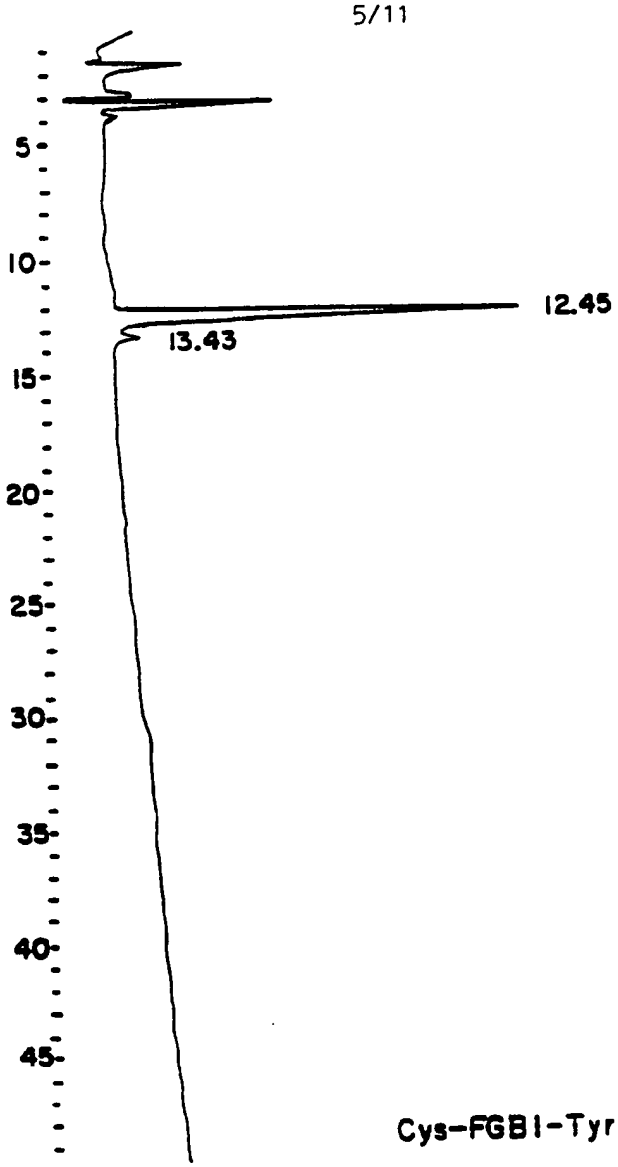
Cys-FGB2-Tyr

FIG.5



FGB2

FIG.6

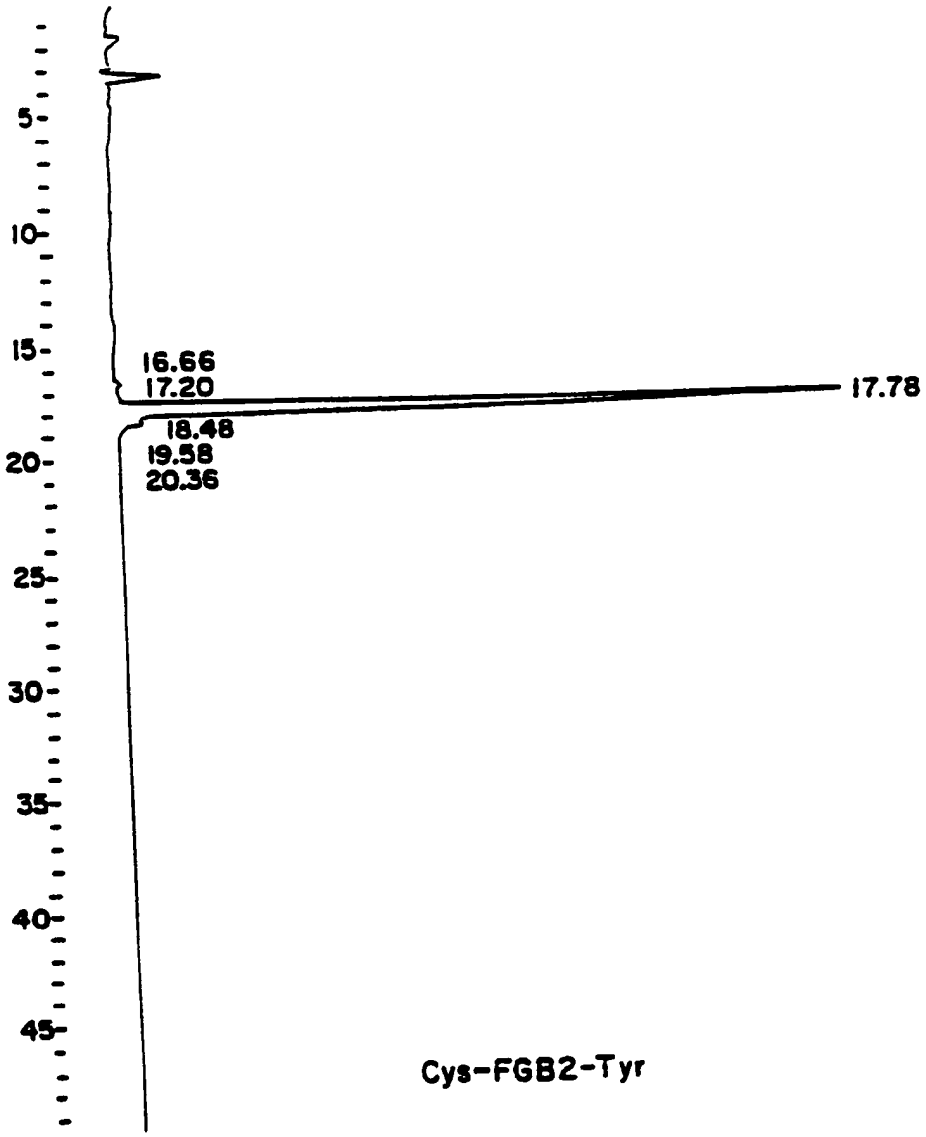


C8 BRWNLE 300A 7UM 2.1 X 220MM
230UL/MIN A: 0.1% TFA H2O
B: 0.08% TFA 100% CH3CN
GRAD 0 TO 60% 45MIN. 220NM

NO.	RT	AREA	CONC
1	12.45	2799668	96.056
2	13.43	114952	3.943
TOTAL		2914620	100.000

RT=RETENTION TIME IN MINUTES

FIG. 7



C8 BRWNLE 300A 7UM 2.1 X 220MM
230UL/MIN A: 0.1% TFA H2O
B: 0.08% TFA 100% CH3CN
GRAD 0 TO 60% 45 MIN. 220NM

NO.	RT	AREA	CONC
1	16.66	123785	0.641
2	17.20	80688	0.418
3	17.78	18954167	98.287
4	18.48	82311	0.426
5	19.58	23000	0.119
6	20.36	20452	0.106
TOTAL		19284403	100.000

RT= RETENTION TIME IN MINUTES

FIG. 8

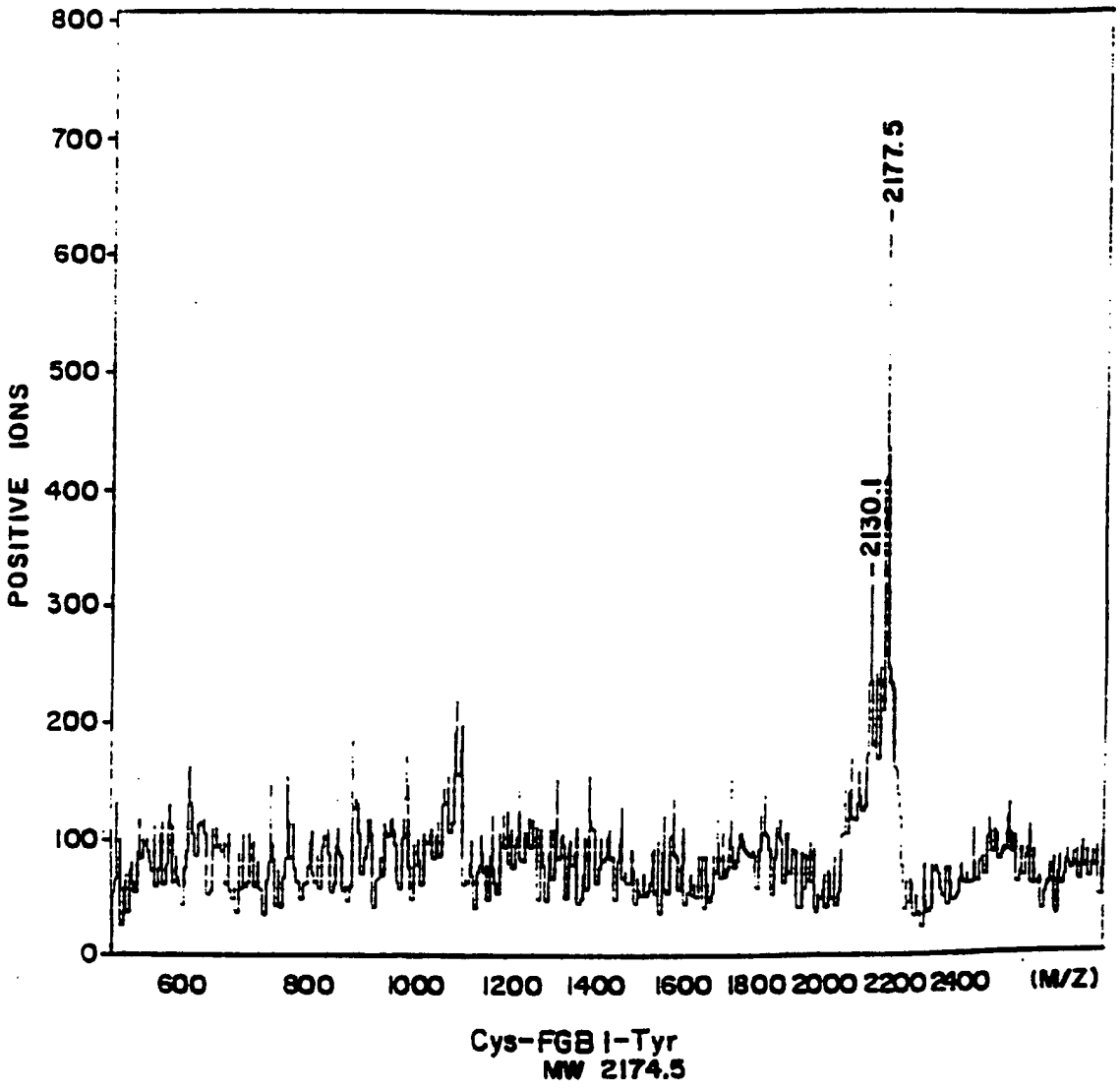

800
700
600
500
400
300
200
100
0
POSITIVE IONS
2177.5
2130.1
600
800
1000
1200
1400
1600
1800
2000
2200
2400
(M/Z)
Cys-FGB 1-Tyr
MW 2174.5

FIG. 9

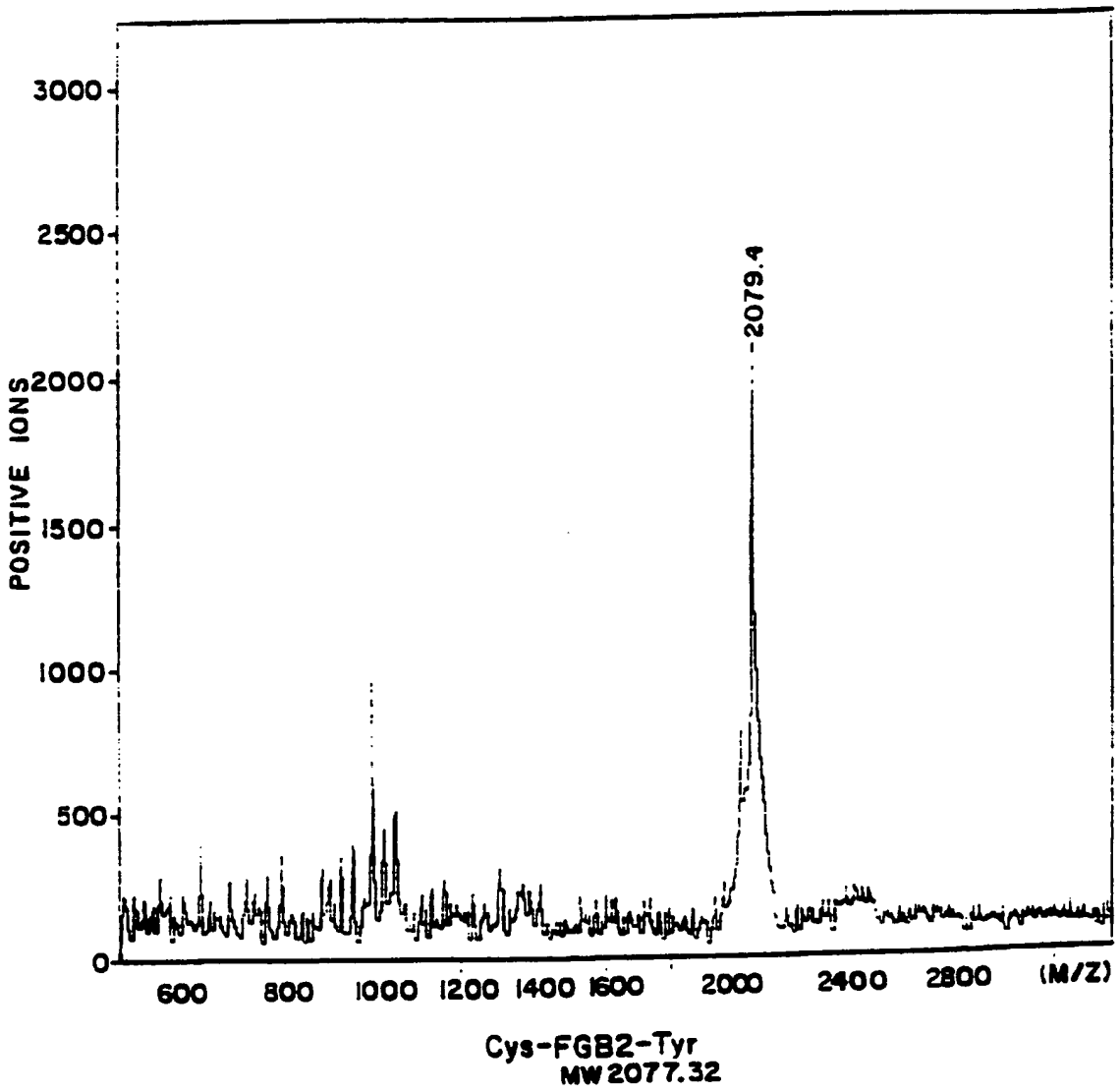

3000
2500
2000
1500
1000
500
0
POSITIVE IONS
2079.4
600
800
1000
1200
1400
1600
2000
2400
2800
(M/Z)
Cys-FGB2-Tyr
MW 2077.32

FIG. 10

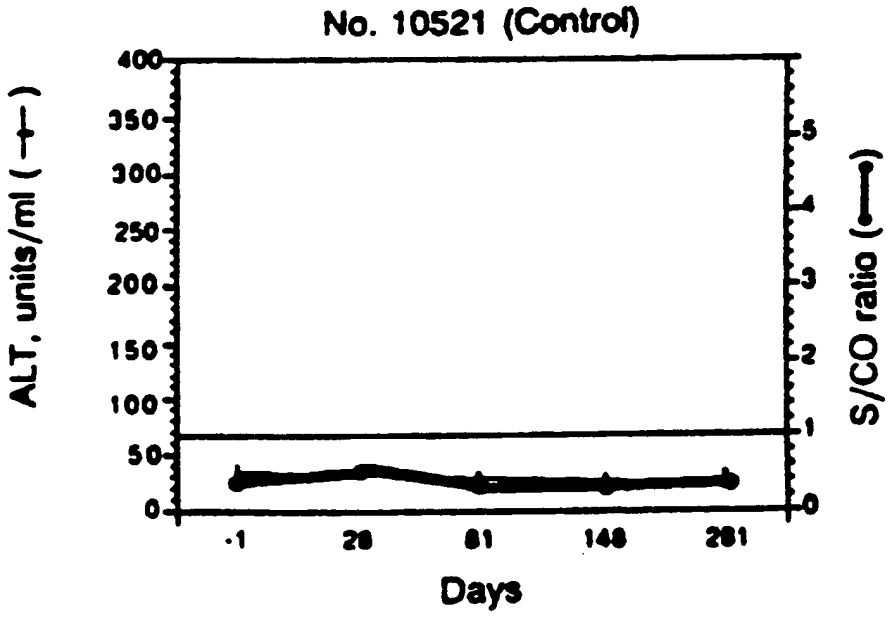

No. 10521 (Control)
ALT, units/ml (—+—)
S/CO ratio (—●—)
400
350
300
250
200
150
100
50
0
5
4
3
2
1
0
-1
28
81
148
281
Days

FIG. 11A

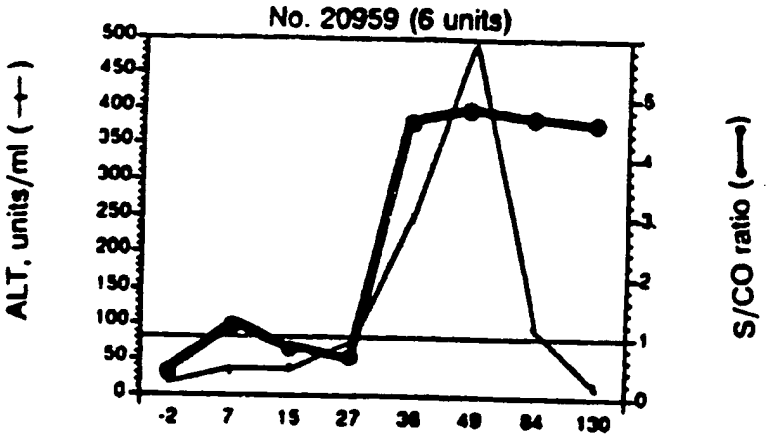


FIG. 11B

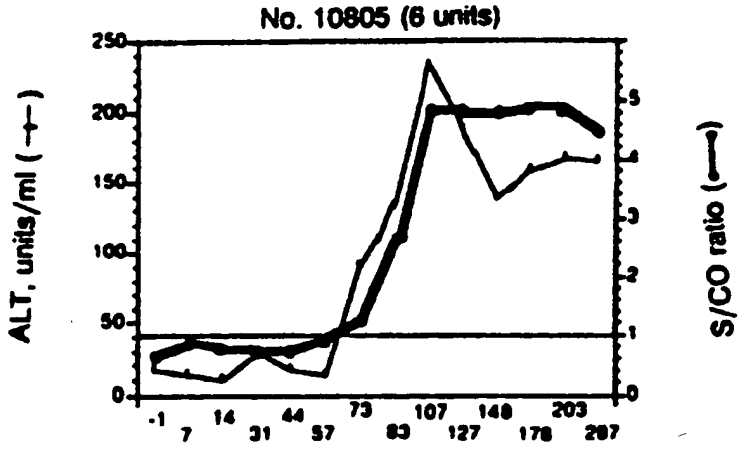


FIG. 11D

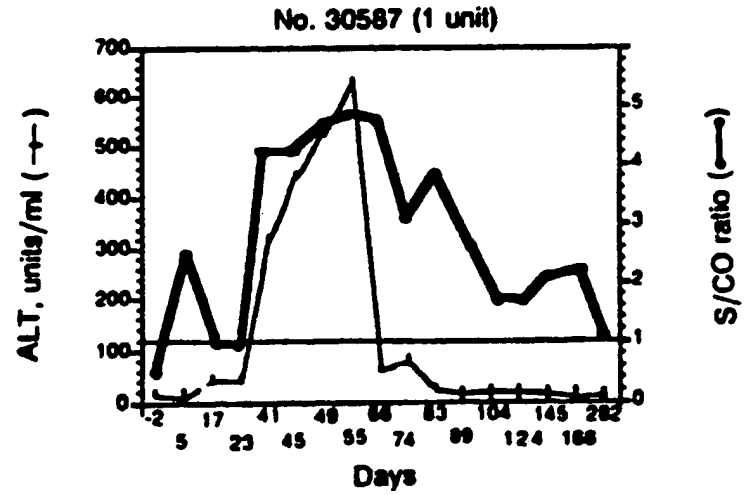


FIG. 11F

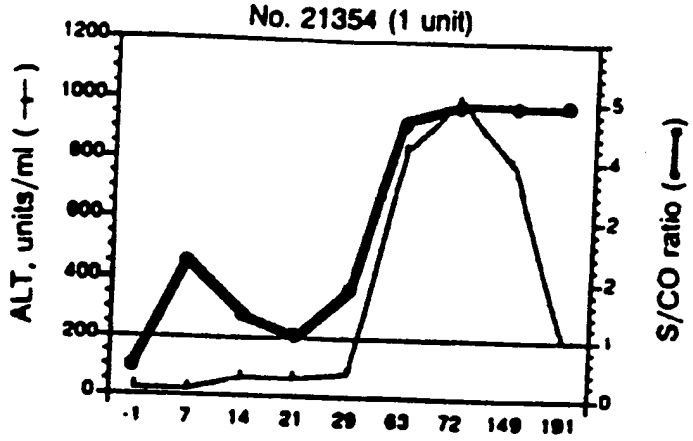


FIG. 11C

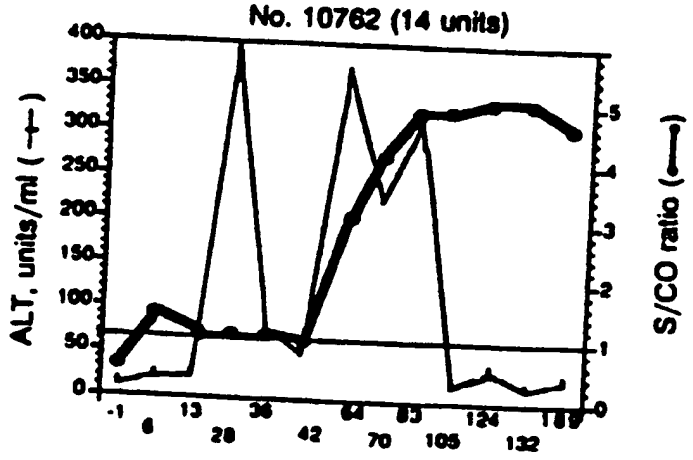


FIG. 11E

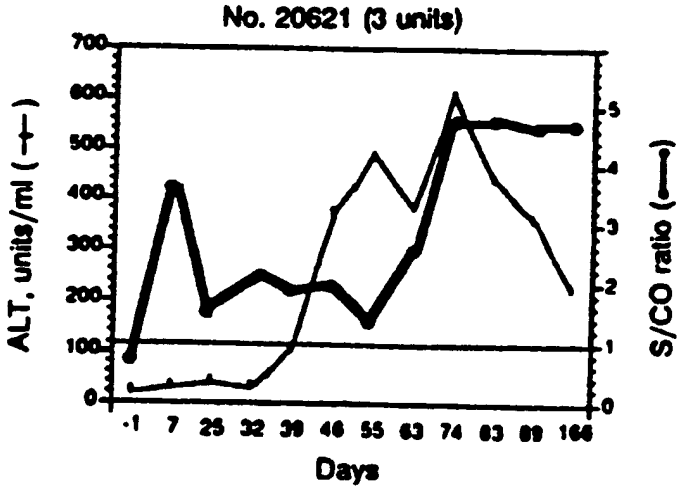


FIG. 11G